FORM PTO 1390
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Rec'd PCT/PTO 12 APR 2001

ATTORNEY'S DOCKET NUMBER: 0459-0571P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/807345

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DK99/00562	October 15, 1999	October 15, 1998

TITLE OF INVENTION
AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE IN A *

APPLICANT(S) FOR DO/EO/US
ARKHAMMAR, Per O.G.; TERRY, Bernard Robert; SCUDDER, Kurt Marshall; BJORN, Sara Petersen **

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/23615
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is transmitted herewith.
   - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98-International Search Report (PCT/ISA/210)
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
    1.) Sixty-eight (68) pages of Sequence Listing
    2.) PCT Request (PCT/RO/101)
    3.) PCT Substitute Claims Letter w/ International Preliminary Examination Report (PCT/IPEA/409) and claims
    4.) Eighteen (18) sheets of Formal Drawings

*CELLULAR RESPONSE
**THASTRUP, Ole; HAGEL, Grith

JC02 Rec'd PCT/PTO 12 APR 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/807345	PCT/DK99/00562	0459-0571P

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). . . . . **$100.00** **ENTER APPROPRIATE BASIC FEE AMOUNT =**	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 - 20 =	0	X $18.00	$ 0	
Independent Claims	1 - 3 =	0	X $80.00	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		None	+ $270.00	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 860.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 430.00	
SUBTOTAL =				$ 0	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 470.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 470.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. ___________ in the amount of $_______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
(703)205-8000

Date: April 12, 2001

By ______ #36,623
Leonard R. Svensson, #30,330

/cqc

U9/807345

PATENT
0459-0571P

JC02 Rec'd PCT/PTO 12 APR 2001

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: ARKHAMMAR, Per O.G. et al. Conf.:

Int'l. Appl. No.: PCT/DK99/00562

Appl. No.: New Group:

Filed: April 12, 2001 Examiner:

For: AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE IN A CELLULAR RESPONSE

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

April 12, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/DK99/00562 which has an International filing date of October 15, 1999, which designated the United States of America and was published in English.--

IN THE CLAIMS:

Please amend the claims as follows:

3. (Amended) A method according to claim 1, wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. (Amended) A method according to claim 1, wherein the cells comprise a group of cells contained within a spatial limitation.

5. (Amended) A method according to claim 1, wherein the cells comprises multiple groups of cells contained within multiple spatial limitations.

6. (Amended) A method according to claim 1, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

8. (Amended) A method according to claim 1, wherein the redistribution results in quenching of fluorescence, the quenching being measure as a decrease in the intensity of the fluorescence.

9. (Amended) A method according to claim 1, wherein the redistribution results in energy transfer, the energy transfer being measure as a change in the intensity of the luminescence.

10. (Amended) A method according to claim 1, wherein the intensity of the light being recorded is a function of the fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

11. (Amended) A method according to claim 1, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. (Amended) A method according to claim 1, wherein the fluorescence comes from a fluorophore encoded by and expressed from a nucleotide sequence harboured in the cells.

13. (Amended) A method according to claim 1, wherein the fluorescence comes from a luminescent polypeptide, such as GFP.

14. (Amended) A method according to claim 1, wherein the luminescent polypeptide could be a GFP selected from the group consisting of green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFp, F64L-S65T-GFP, and EGFP.

15. (Amended) A method according to claim 1, wherein the cells are selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells.

17. (Amended) A method according to claim 1, used as a screening program.

20. (Amended) A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to claim 1.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By M J Nell #36,623
Leonard R. Svensson, #30,330

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

LRS/cqc
0459-0571P

Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

Docket No. 0459-0571P

VERSION WITH MARKINGS SHOWING CHANGES MADE

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

3. (Amended) A method according to claim 1 [or 2], wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. (Amended) A method according to [any of claims 1-3]<u>claim 1</u>, wherein the cells comprise a group of cells contained within a spatial limitation.

5. (Amended) A method according to [any of claims 1-4]<u>claim 1</u>, wherein the cells comprises multiple groups of cells contained within multiple spatial limitations.

6. (Amended) A method according to [any of claims 1-5]<u>claim 1</u>, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

8. (Amended) A method according to [any of claims 1-7]claim 1, wherein the redistribution results in quenching of fluorescence, the quenching being measure as a decrease in the intensity of the fluorescence.

9. (Amended) A method according to [any of claims 1-8]claim 1, wherein the redistribution results in energy transfer, the energy transfer being measure as a change in the intensity of the luminescence.

10. (Amended) A method according to [any of claims 1-8]claim 1, wherein the intensity of the light being recorded is a function of the fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

11. (Amended) A method according to [any of claims 1-10]claim 1, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. (Amended) A method according to [any of claims 1-11]claim 1, wherein the fluorescence comes from a fluorophore encoded by and expressed from a nucleotide sequence harboured in the cells.

13. (Amended) A method according to [any of the preceding claims]claim 1, wherein the fluorescence comes from a luminescent polypeptide, such as GFP.

14. (Amended) A method according to [any of the preceding claims]claim 1, wherein the luminescent polypeptide could be a GFP selected from the group consisting of green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFp, F64L-S65T-GFP, and EGFP.

15. (Amended) A method according to [any of claims 1-14]claim 1, wherein the cells are selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells.

17. (Amended) A method according to [any of claims 1-16]claim 1, used as a screening program.

20. (Amended) A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to [any of claims 1-19]claim 1.

#3

BOX SEQUENCE
PATENT
0459-0571P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	ARKHAMMAR, Per O.G. et al.	Conf.:	6261
Appl. No.:	09/807,345	Group:	Unassigned
Filed:	April 12, 2001	Examiner:	Unassigned
For:	AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE		

AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

August 14, 2001

Sir:

In response to the U.S. Patent Office Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Disclosures dated May 14, 2001, the period for response having been extended one (1) month to August 14, 2001, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please replace the paragraph beginning on page 40, line 12 with the following amended paragraph:

--To construct the PKAc-F64L-S65T-GFP fusion, convenient restriction endonuclease sites were introduced into the cDNAs encoding murine PKAc (Gen Bank Accession number: M12303) and F64L-S65T-GFP (sequence disclosed in

WO 97/11094) by polymerase chain reaction (PCR). The PCR reactions were performed according to standard protocols with the following primers:

5'PKAc:

TTggACACAAgCTTTggACACCCTCAggATATgggCAACgCCgCCgCCgCCA Ag (SEQ ID NO:19),

3'PKAc:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCAAACTCAgTAAACTCCTTgCCACA C(SEQ ID NO:20)

5'GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTT C(SEQ ID NO:21)

3'GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT (SEQ ID NO:22).--

Please replace the paragraph beginning on page 44, line 32 with the following amended paragraph:

--**EXAMPLE 2 Probe for detection of PKC activity**
Construction of PKC-GFP fusion:

The probe was constructed by ligating two restriction enzyme treated polymerase chain reaction (PCR) amplification products of the cDNA for murine PKCα (GenBank Accession number: M25811) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) respectively. Taq® polymerase and the following oligonucleotide primers were used for PCR;

5'mPKCα:

TTggACACAAgCTTTggACACCCTCAggATATggCTgACgTTTACCCggCCA ACg (SEQID NO:23)

3'mPKCα:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCTACTgCACTTTgCAAgATTgggT gC (SEQ ID NO:24),

5'F64L-S65T-GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTT C (SEQ ID NO:25),

3'F64L-S65T-GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT (SEQ ID NO:26).--

Please replace the paragraph beginning on page 47, line 13 with the following amended paragraph:

--The human Erk1 gene (GenBank Accession number: X60188) was amplified using PCR according to standard protocols with primers

Erk1-top

5'-TAGAATTCAACCATGGCGGCGGCGGCGGCG (SEQ ID NO:27)-3'

and Erk1-bottom/+stop

5'-TAGGATCCCTAGGGGGCCTCCAGCACTCC (SEQ ID NO:28)-3'.

The PCR product was digested with restriction enzymes EcoR1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with EcoR1 and BamH1. This produces an EGFP-Erk1 fusion (SEQ ID NOs: 5 and 6) under the control of a CMV promoter.--

Please replace the paragraph beginning on page 48, line 14 with the following amended paragraph:

--Smad 2, a signal transducer, is a component of a signalling pathway that is induced by some members of the TGFbeta family of cytokines.

a) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:29)-3'

and Smad2-bottom/+stop

5'-GTGGTACCTTATGACATGCTTGAGCAACGCAC (SEQ ID NO:30)-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-C1 (Clontech; Palo Alto; GenBank Accession number U55763) digested with EcoR1 and Acc65I. This produces an EGFP-Smad2 fusion (SEQ ID NOs: 7 and 8) under the control of a CMV promoter.

b) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:31)-3'

and Smad2-bottom/-stop

5'-GTGGTACCCATGACATGCTTGAGCAACGCAC (SEQ ID NO:32)-3'.--

Please replace the paragraph beginning on page 49, line 16 with the following amended paragraph:

--**EXAMPLE 5 Probes for detection of VASP redistribution.**

Useful for monitoring signalling pathways involving rearrangement of cytoskeletal elements, e.g. to identify compounds which modulate the activity of the pathway in living cells. VASP, a phosphoprotein, is a component of cytoskeletal structures, which redistributes in response to signals that affect focal adhesions.

The human VASP gene (GenBank Accession number: Z46389) was amplified using PCR according to standard protocols with primers

VASP-top

5'-GGGAAGCTTCCATGAGCGAGACGGTCATC (SEQ ID NO:33)-3'

and VASP-bottom/+stop

5'-CCCGGATCCTCAGGGAGAACCCCGCTTC (SEQ ID NO:34)-3'.--

Please replace the paragraph beginning on page 50, line 4 with the following amended paragraph:

--**EXAMPLE 7 Probes for detection of NFkappaB redistribution.**
Useful for monitoring signalling pathways leading to activation of NFkappaB, e.g. to identify compounds which modulate the activity of the pathway in living cells.

NFkappaB, an activator of transcription, is a component of signalling pathways that are responsive to a varity of inducers including cytokines, lymphokines, and some immunosuppressive agents.

a) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:35)-3'

and NFkappaB-bottom/+stop

5'-GTGGATCCTTAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:36)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Xho1 and BamH1. This produces an EGFP-NFkappaB fusion (SEQ ID NOs:13 and 14) under the control of a CMV promoter.

b) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:37)-3'

and NFkappaB-bottom/-stop

5'-GTGGATCCAAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:38)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Xho1 and BamH1. This produces an NFkappaB-EGFP fusion (SEQ ID NOs: 15 and 16) under the control of a CMV promoter.--

Please replace the paragraph beginning on page 54, line 1 with the following amended paragraph:

--**EXAMPLE 11 Probes for detection of PKCβ1 redistribution.**

Useful for monitoring signalling pathways involving Protein Kinase C, e.g. to identify compounds which modulate the activity of the pathway in living cells. PKCbeta1, a serine/threonine protein kinase, is closely related to PKCalpha and PKCbeta2 but not identical; it is a component of a signalling pathway which is activated by elevation of intracellular calcium concomitant with an increase in diacylglycerol species.

a) The human PKCbeta1 gene (GenBank Accession number: X06318) was amplified using PCR according to standard protocols with primers

PKCβ1-top

GTCTCGAGGCAAGATGGCTGACCC (SEQ ID NO:39)

and PKCβ1-bottom

GTGGATCCCTACACATTAATGACAAACTCTGGG (SEQ ID NO:40).--

Please replace the Sequence Listing filed April 12, 2001 located immediately after the claims with substitute Sequence Listing enclosed herewith.

REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a substitute Sequence Listing to be inserted into the specification as indicated above. The substitute Sequence Listing in no way introduces new matter into the specification.

Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the substitute Sequence Listing. The disk copy of the substitute Sequence Listing, file "0459-0571P.ST25", is identical to the paper copy, except that it lacks formatting.

The substitute Sequence Listing includes primer sequences found in the Specification as filed that were not made part of the original Sequence Listing. The amendments to the Specification are being made to reference these sequences by their SEQ ID NOS. These amendments are editorial in nature and do not constitute new matter.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Pursuant to C.F.R. §§1.17 and 1.136(a), the Applicant respectfully petitions for a one (1) month extension of time for filing a response in connection with the present application and the required fee of $110.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By #36,623
Leonard R. Svensson, #30,330

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

LRS/KW
0459-0571P

Attachments: Paper and disk copy of Sequence Listing
Copy of Notice to Comply
Copy of Version with Markings to Show Changes Made

VERSION WITH MARKING TO SHOW CHANGES MADE

The paragraph beginning on page 40, line 12 has been amended as follows:

To construct the PKAc-F64L-S65T-GFP fusion, convenient restriction endonuclease sites were introduced into the cDNAs encoding murine PKAc (Gen Bank Accession number: M12303) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) by polymerase chain reaction (PCR). The PCR reactions were performed according to standard protocols with the following primers:

5'PKAc:

TTggACACAAgCTTTggACACCCTCAggATATgggCAACgCCgCCgCCgCCAAg (SEQ ID NO:19),

3'PKAc:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCAAACTCAgTAAACTCCTTgCCACAC(SEQ ID NO:20)

5'GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTTC(SEQ ID NO:21)

3'GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT(SEQ ID NO:22).

The paragraph beginning on page 44, line 32 has been amended as follows:

EXAMPLE 2 Probe for detection of PKC activity

Construction of PKC-GFP fusion:

The probe was constructed by ligating two restriction enzyme treated polymerase chain reaction (PCR) amplification products of the cDNA for murine PKCα (GenBank Accession number:

M25811) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) respectively. Taq® polymerase and the following oligonucleotide primers were used for PCR;

5'mPKCα: TTggACACAAgCTTTggACACCCTCAggATATggCTgACgTTTACCCggCCAACg (SEQID NO:23)

3'mPKCα:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCTACTgCACTTTgCAAgATTgggTgC (SEQ ID NO:24),

5'F64L-S65T-GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTTC (SEQ ID NO:25),

3'F64L-S65T-GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT (SEQ ID NO:26).

The paragraph beginning on page 47, line 13 has been amended as follows:

The human Erk1 gene (GenBank Accession number: X60188) was amplified using PCR according to standard protocols with primers

Erk1-top

5'-TAGAATTCAACCATGGCGGCGGCGGCGGCG (SEQ ID NO:27)-3'

and Erk1-bottom/+stop

5'-TAGGATCCCTAGGGGGCCTCCAGCACTCC (SEQ ID NO:28)-3'.

The PCR product was digested with restriction enzymes EcoR1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with EcoR1 and

BamH1. This produces an EGFP-Erk1 fusion (SEQ ID NOs: 5 and 6) under the control of a CMV promoter.

The paragraph beginning on page 49, line 16 has been amended as follows:

Smad 2, a signal transducer, is a component of a signalling pathway that is induced by some members of the TGFbeta family of cytokines.

a) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:29)-3'

and Smad2-bottom/+stop

5'-GTGGTACCTTATGACATGCTTGAGCAACGCAC (SEQ ID NO:30)-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-C1 (Clontech; Palo Alto; GenBank Accession number U55763) digested with EcoR1 and Acc65I. This produces an EGFP-Smad2 fusion (SEQ ID NOs: 7 and 8) under the control of a CMV promoter.

b) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:31)-3'

and Smad2-bottom/-stop

5'-GTGGTACCCATGACATGCTTGAGCAACGCAC (SEQ ID NO:32)-3'.

The paragraph beginning on page 48, line 14 has been amended as follows:

EXAMPLE 5 Probes for detection of VASP redistribution.

Useful for monitoring signalling pathways involving rearrangement of cytoskeletal elements, e.g. to identify compounds which modulate the activity of the pathway in living cells. VASP, a phosphoprotein, is a component of cytoskeletal structures, which redistributes in response to signals that affect focal adhesions.

The human VASP gene (GenBank Accession number: Z46389) was amplified using PCR according to standard protocols with primers

VASP-top

5'-GGGAAGCTTCCATGAGCGAGACGGTCATC (SEQ ID NO:33)-3'

and VASP-bottom/+stop

5'-CCCGGATCCTCAGGGAGAACCCCGCTTC (SEQ ID NO:34)-3'.

The paragraph beginning on page 50, line 4 has been amended as follows:

EXAMPLE 7 Probes for detection of NFkappaB redistribution.

Useful for monitoring signalling pathways leading to activation of NFkappaB, e.g. to identify compounds which modulate the activity of the pathway in living cells.

NFkappaB, an activator of transcription, is a component of signalling pathways that are responsive to a varity of inducers including cytokines, lymphokines, and some immunosuppressive agents.

a) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers

NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:35)-3'

and NFkappaB-bottom/+stop

5'-GTGGATCCTTAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:36)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Xho1 and BamH1. This produces an EGFP-NFkappaB fusion (SEQ ID NOs:13 and 14) under the control of a CMV promoter.

b) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers

NFkappaB-top

5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:37)-3'

and NFkappaB-bottom/-stop

5'-GTGGATCCAAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:38)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Xho1 and BamH1. This produces an NFkappaB-EGFP fusion (SEQ ID NOs: 15 and 16) under the control of a CMV promoter.

The paragraph beginning on page 54, line 1 has been amended as follows:

EXAMPLE 11 Probes for detection of PKCβ1 redistribution.

Useful for monitoring signalling pathways involving Protein Kinase C, e.g. to identify compounds which modulate the activity of the pathway in living cells.

PKCbeta1, a serine/threonine protein kinase, is closely related to PKCalpha and PKCbeta2 but not identical; it is a component of a signalling pathway which is activated by elevation of intracellular calcium concomitant with an increase in diacylglycerol species.

a) The human PKCbeta1 gene (GenBank Accession number: X06318) was amplified using PCR according to standard protocols with primers

PKCβ1-top

GTCTCGAGGCAAGATGGCTGACCC (SEQ ID NO:39)

and PKCβ1-bottom

GTGGATCCCTACACATTAATGACAAACTCTGGG (SEQ ID NO:40).

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AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE.

SUMMARY OF THE INVENTION

The present invention relates to an improved method and tools for extracting quantitative information relating to an influence on a cellular response, in particular an influence caused by contacting or incubating the cell with a substance influencing a cellular response, wherein the cellular response is manifested in redistribution of at least one component in the cell. In particular, the invention relates to an improved method for extracting the quantitative information relating to an influence on an intracellular pathway involving redistribution of at least one component associated with the pathway. The method of the invention may be used as a very efficient procedure for testing or discovering the influence of a substance on a physiological process, for example in connection with screening for new drugs, testing of substances for toxicity, identifying drug targets for known or novel drugs. In particular, the present invention relates to an improved method for parallelisation of the testing procedure so that a large number of substances can be tested simultaneously using commercially available instrumentation. The invention also describes several ways of contacting the cells with a substance influencing a cellular response and modifications made to the actual cells before, during or after contacting the cells with these substances as to improve the applicability and use of the method for extracting quantitative information relating to influence on an intracellular pathway in a highly parallel fashion. Other valuable uses of the method and technology of the invention will be apparent to the skilled person on the basis of the following disclosure. In a particular embodiment of the invention, the present invention relates to a method of detecting intracellular translocation or redistribution of biologically active polypeptides, preferably an enzyme, affecting intracellular processes, and a DNA construct and a cell for use in the method.

BACKGROUND OF THE INVENTION

Intracellular pathways are tightly regulated by a cascade of components that undergo modulation in a temporally and spatially characteristic manner. Several disease states can be attributed to altered activity of individual signalling components (i.e. protein

kinases, protein phosphatases, transcription factors). These components therefore render themselves as attractive targets for therapeutic intervention.

Protein kinases and phosphatases are well-described components of several intracellular signalling pathways. The catalytic activity of protein kinases and phosphatases are assumed to play a role in virtually all regulatable cellular processes. Although the involvement of protein kinases in cellular signalling and regulation have been subjected to extensive studies, detailed knowledge on e.g. the exact timing and spatial characteristics of signalling events is often difficult to obtain due to lack of a convenient technology.

The measurement of the activity of intracellular enzymes, such as kinases and phosphatases, can be performed by well-established procedures, both manually and in various automated forms, at throughput rates which make these measurements useful in the search for new drug candidates. In addition to measures of activity, measures of the distribution of these and other enzymes in the cell has proven useful, and established techniques exist for this type of measurement as well. Protein kinases often show a specific intracellular distribution before, during and after activation. Monitoring the translocation processes and/or redistribution of individual protein kinases or subunits thereof is thus likely to be indicative of their functional activity. A connection between translocation and catalytic activation has been shown for protein kinases like the diacyl glycerol (DAG)-dependent protein kinase C (PKC), the cAMP-dependent protein kinase (PKA) [(DeBernardi *et al.*1996)] and the mitogen-activated-protein kinase Erk-1 [(Sano *et al.*1995)]. Such methods of detection of intracellular localisation/activity of protein kinases and phosphatases include immunoprecipitation, Western blotting and immunocytochemical detection.

One aspect of the function of intracellular enzymes which has not been characterised so thoroughly is the redistribution of those enzymes. The importance of subcellular redistribution of enzymes as a mechanism of enzyme specificity, and of the general importance of the measurement of subcellular redistribution as a tool for identifying novel drug targets and searching for drug candidates which influence those targets, is disclosed in: A METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE ,the contents of which

were part of the priority application, and which, as WO9845704 has been published during the priority year, are hereby incorporated herein by reference.

While the redistribution of subcellular components is known to be important, the measurement of this phenomenon in real time has not been widely exploited. This is primarily due to the lack of a suitable technique. There is essentially only one direct technique: the microscopic imaging of cells in which the subcellular component of interest has been labelled in such a way that it can be visualised and recorded by the microscopic imaging system, using for example a video or scientific CCD camera and appropriate software for collecting and storing the images. Novel ways of monitoring specific modulation of intracellular pathways in intact, living cells is assumed to provide new opportunities in drug discovery, functional genomics, toxicology, patient monitoring etc.

Recently it was discovered that Green Fluorescent Protein (GFP) expressed in many different cell types, including mammalian cells, became highly fluorescent [(Chalfie *et al.* 1994)]. WO95/07463 describes a cell capable of expressing GFP and a method for detecting a protein of interest in a cell based on introducing into a cell a DNA molecule having DNA sequence encoding the protein of interest linked to DNA sequence encoding a GFP such that the protein produced by the DNA molecule will have the protein of interest fused to the GFP, then culturing the cells in conditions permitting expression of the fused protein and detecting the location of the fluorescence in the cell, thereby localizing the protein of interest in the cell. However, examples of such fused proteins are not provided, and the use of fusion proteins with GFP for detection or quantitation of translocation or redistribution of biologically active polypeptides affecting intracellular processes upon activation, such as proteins involved in signalling pathways, e.g. protein kinases or phosphatases, has not been suggested. WO 95/07463 further describes cells useful for the detection of molecules, such as hormones or heavy metals, in a biological sample, by operatively linking a regulatory element of the gene which is affected by the molecule of interest to a GFP, the presence of the molecules will affect the regulatory element which in turn will affect the expression of the GFP. In this way the gene encoding GFP is used as a reporter gene in a cell which is constructed for monitoring the presence of a specific molecular identity.

Green Fluorescent Protein has been used in an assay for the detection of translocation of the glucocorticoid receptor (GR) [(Carey, KL *et al.*1996)]. A GR-S65TGFP fusion has been used to study the mechanisms involved in translocation of the glucocorticoid receptor (GR) in response to the agonist dexamethasone from the cytosol, where it is present in the absence of a ligand, through the nuclear pore to the nucleus where it remains after ligand binding. The use of a GR-GFP fusion enables real-time imaging and quantitation of nuclear/cytoplasmic ratios of the fluorescence signal. A similar genetic construct has been used to follow and quantify dexamethasone induced translocation of GR to the nucleus in HeLa cells [(Guiliano, K.A *et al.* 1997)] in a system called Array Scan™ (WO 97/45730) designed for automated drug screening. Recently, several other investigators have demonstrated that tagging a specific protein (or part of a protein) involved in an intracellular signalling pathway with GFP provides a new means to measure and quantify the influence of substances on this pathway. The concept has been shown to work both for cytoplasmic to nuclear translocation of the androgen receptor [(Georget V *et al.* 1997)] and transcription factors such as NF-ATc [(Beals CR *et al. 1997*)] in analogy with what has already been described for GR above. Another relevant example is a β-arrestin – GFP construct that was shown to report on activation of G-protein coupled receptors by translocating from the cytosol to the plasma membrane [(Barak LS *et al.* 1997)]. Finally, it has also been demonstrated that attaching GFP to a smaller part of a protein like the pleckstrin homology domain of phospholipase C δ 1 [(Stauffer TP *et al.* 1998)] and a cysteine-rich domain of PKC γ [(Oancea E *et al.* 1998)] can be used to report on an influence from a substance by quantifying their redistribution within the cells during activation of the specific signalling pathway to which they belong.

Many currently used screening programmes designed to find compounds that affect protein kinase activity are based on measurements of kinase phosphorylation of artificial or natural substrates, receptor binding and/or reporter gene expression. The interest in fluorescence measurements as the basis for future high-throughput drug screening has however increased dramatically over the last few years [(Silverman L *et al.* 1998)]. Of particular interest to the present invention is a scanning laser imager for rapid screening of fluorescence changes in living cells [(Schroeder K & Neagle B 1996)] currently offered commercially by Molecular Devices, Inc. as the FLIPR™.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an important new dimension in the investigation of cellular systems involving redistribution in that the invention provides quantification of the redistribution responses or events caused by an influence, typically contact with a chemical substance or mixture of chemical substances, but also changes in the physical environment, in a massively parallel fashion. The quantification makes it possible to set up meaningful relationships, expressed numerically, or as curves or graphs, between the influences (or the degree of influences) on cellular systems and the redistribution response. This is highly advantageous because, as has been found, the quantification can be achieved in both a fast and reproducible manner, and - what is perhaps even more important - the systems which become quantifiable utilising the method of the invention are systems from which enormous amounts of new information and insight can be derived.

The present screening assays have the distinct advantage over other screening assays, e.g., receptor binding assays, enzymatic assays, and reporter gene assays, in providing a system in which biologically active substances with completely novel modes of action, e.g. inhibition or promotion of redistribution/translocation of a biologically active polypeptide as a way of regulating its action rather than inhibition/activation of enzymatic activity, can be identified in a way that insures very high selectivity to the particular isoform of the biologically active polypeptide and further development of compound selectivity versus other isoforms of the same biologically active polypeptide or other components of the same signalling pathway.

In one of its broadest aspects, the invention relates to an improved method, with higher throughput compared to previous methods, for extracting quantitative information relating to an influence on a cellular response, the method comprising recording variation, caused by the influence on mechanically intact living cells, in spatially distributed light emitted from a luminophore, the luminophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence, the association resulting in a modulation of the luminescence characteristics of the luminophore, detecting and recording the variation in spatially distributed light from the luminophore as

a change in fluorescence intensity using an instrument designed to measure changes in fluorescence intensity, and processing the recorded variation in the spatially distributed light to provide quantitative information correlating the spatial distribution or change in the spatial distribution to the degree of the influence. In one aspect of the present invention the mechanically intact living cell is permeabilised at some time after the influence has begun but during or before the actual experimental recording. In another aspect, the present invention relates to an improved method for extracting quantitative information relating to an influence on a cellular response, the method comprising recording variation, caused by the influence on permeabilised living cells, in spatially distributed light emitted from a luminophore, the luminophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence, the association resulting in a modulation of the luminescence characteristics of the luminophore, detecting and recording the spatially distributed light from the luminophore as a change in fluorescence intensity using an instrument designed to measure changes in fluorescence intensity, and processing the recorded variation in the spatially distributed light to provide quantitative information correlating the spatial distribution or change in the spatial distribution to the degree of the influence. In a preferred embodiment of the invention the luminophore, which is present in the cells, is capable of being redistributed by modulation of an intracellular pathway, in a manner which is related to the redistribution of at least one component of the intracellular pathway. In another preferred embodiment of the invention, the luminophore is a fluorophore.

Typically the cell and/or cells are mechanically intact and alive throughout the experiment. In another embodiment of the invention, the cells are fixed at a point in time after the application of the influence at which the response has been predetermined to be significant, and the recording is made at an arbitrary later time. In another embodiment the cell and/or cells are mechanically intact and alive throughout the experiment but are mechanically or chemically disrupted or permeabilised as the initial step of experimental analysis. In another aspect of the invention the cells have their plasma membrane permanently and stably permeabilised before the initiation of the experiment in such a way that the plasma membrane stays permeable during the experiment. This allows the components of intracellular pathways to be contacted by

substances that are not normally permeating the cell plasma membrane such as peptides, proteins and hydrophilic organic compounds..

The mechanically intact or permeabilised living cells could be selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells. These cells are incubated at a temperature of 30°C or above, preferably at a temperature of from 32°C to 39°C, more preferably at a temperature of from 35°C to 38°C, and most preferably at a temperature of about 37°C during the time period over which the influence is observed. In one aspect of the invention the mechanically intact or permeabilised living cell is part of a matrix of identical or non-identical cells. In one embodiment of the invention the cells comprise a group or groups of cells contained within a spatial limitation or spatial limitations. In one embodiment, the cells comprise multiple groups of cells that are qualitatively the same but subjected to different influences. In another embodiment, the cells comprise multiple groups of cells that are qualitatively different but subjected to the same influence.

In one embodiment of the invention the spatial limitations are domains defined on a substrate on which the cells are present. The spatial limitations may be arranged in one or more arrays on a common carrier. The spatial limitations may be wells in a plate of microtiter type, such that 96, 384. 864 and 1536 wells are situated on the common carrier. In another embodiment the spatial limitations are wells in a plate of a format different from the microtiter type. In one embodiment of the invention the domains are established by the presence of the cells on the substrate in a pattern that defines the domains. In another aspect of the invention, the domains are instead established by the spatial pattern or array of the influence or influences as it/they are applied to or contacted by the cells. This aspect is thoroughly disclosed in "Method and apparatus for high density format screening for bioactive molecules" the contents of which were part of the priority application, and which. as WO99/35496 has been published during the priority year, are hereby incorporated herein by reference. Briefly, in this aspect of the invention the mechanically intact or permeabilised living cells are part of a continuous or discontinuous sheet of cells cultured on an optically clear flat surface typically optimised for cell culture. The optically clear and flat surface may be a porous membrane that may allow cellular processes to grow through the membrane pores and may allow directed capillary flow of fluid through the pores.

A cell used in the present invention should contain a nucleic acid construct encoding a fusion polypeptide as defined herein and be capable of expressing the sequence encoded by the construct. The cell is a eukaryotic cell selected from the group consisting of fungal cells, such as yeast cells: invertebrate cells including insect cells; vertebrate cells such as mammalian cells. The preferred cells are mammalian cells.

In another aspect of the invention the cells could be from an organism carrying in at least one of its component cells a nucleic acid sequence encoding a fusion polypeptide as defined herein and be capable of expressing said nucleic acid sequence. The organism is selected from the group consisting of unicellular and multicellular organisms, such as a mammal.

The luminophore is the component that allows the redistribution to be visualised and/or recorded by emitting light in a spatial distribution related to the degree of influence. The term redistribution is intended to cover all aspects of a change in spatial location, such as a translocation of the luminophore or other components. In one embodiment of the invention, the luminophore is capable of being redistributed in a manner that is physiologically relevant to the degree of the influence. In another embodiment, the luminophore is capable of associating with a component that is capable of being redistributed in a manner that is physiologically relevant to the degree of the influence. In another embodiment, a correlation between the redistribution of the luminophore and the degree of the influence could be determined experimentally. In a preferred aspect of the invention, the luminophore is capable of being redistributed in substantially the same manner as the at least one component of an intracellular pathway. In another embodiment of the invention, the luminophore is capable of being quenched upon spatial association with a component that is redistributed by modulation of the pathway, the quenching being measured as a change in the intensity of the luminescence. In another embodiment of the invention, the luminophore is stationary but may have a certain spatial distribution, and interacts with at least one component that is capable of being redistributed in a manner which is physiologically relevant to the degree of the influence, in such a way that one or more luminescence characteristics of the luminophore is/are modulated as the component moves closer to, or farther from, the luminophore.

The luminophore could be a fluorophore. In a preferred embodiment of the invention, the luminophore is a polypeptide encoded by and expressed from a nucleotide sequence

harboured in the cells. The luminophore could be a hybrid polypeptide comprising a fusion of at least a portion of each of two polypeptides one of which comprises a luminescent polypeptide and the other one of which comprises a biologically active polypeptide, as defined herein.

The luminescent polypeptide could be a GFP as defined herein or could be selected from the group consisting of green fluorescent proteins having the F64L mutation as defined herein such as F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP. The GFP could be N- or C-terminally tagged, optionally via a peptide linker, to the biologically active polypeptide or a part or a subunit thereof. The fluorescent probe could be a component of an intracellular signalling pathway. The probe is coded for by a nucleic acid construct.

In one aspect of the invention the pathway of investigation is an intracellular signalling pathway.

In a preferred embodiment of the invention, the influence could be contact between the group or groups of mechanically intact or permeabilised living cells and a chemical substance, and/or incubation of the group or groups of mechanically intact or permeabilised living cells with a chemical substance in solution. In one aspect of the invention that is thoroughly described in "Method and apparatus for high density format screening for bioactive molecules" the contents of which were part of the priority application, and which, as WO99/35496 has been published during the priority year, are hereby incorporated herein by reference, the chemical substances are attached to an underlying matrix. In this aspect, the chemical substances may also be produced and secreted from, or attached to the plasma membrane surfaces of, a sheet of genetically engineered cells. In this aspect of the invention the chemical substances may also have been separated two-dimensionally in a non-denaturing gel using electrophoresis and the gel is directly put in close proximity or direct contact with the mechanically intact or permeabilised living cells so that the chemical substances can contact the cells through diffusion or convection.

The influence will modulate the intracellular processes. In one aspect the modulation could be an activation of the intracellular processes. In another aspect the modulation could be a deactivation of the intracellular processes. In yet another aspect, the

influence could inhibit or promote the redistribution without directly affecting the metabolic activity of the component of the intracellular processes.

In one embodiment the invention is used to establish a dose-response relationship for one or many chemical substances. In one embodiment the invention is used as a basis for a screening program, where the effect of unknown influences such as a compound library, can be compared to influence of known reference compounds under standardised conditions.

In addition to the intensity, there are several parameters of fluorescence or luminescence that can be modulated by the effect of the influence on the underlying cellular phenomena, and can therefore be used in the invention. Some examples are resonance energy transfer, fluorescence lifetime, polarisation, and wavelength shift. Each of these methods requires a particular kind of filter in the emission light path to select the component of the light desired and reject other components. The recording of property of light could be in the form of an ordered array of values such as a CCD array or a vacuum tube device such as a vidicon. In addition, the translational mobility, or freedom of movement, of the luminophore attached to the protein of interest can be an important property affected by the influence on the underlying cellular phenomena, and can therefore be used in the invention.

In one embodiment of the invention, the spatially distributed light emitted by a luminophore is detected by a change in the resonance energy transfer between the luminophore and another luminescent entity capable of delivering energy to the luminophore, each of which has been selected or engineered to become part of, bound to or associated with particular components of the intracellular pathway. In this embodiment, either the luminophore or the luminescent entity capable of delivering energy to the luminophore undergoes redistribution in response to an influence. The resonance energy transfer would be measured as a change in the intensity of emission from the luminophore, preferably sensed by a single channel photodetector that responds only to the average intensity of the luminophore in a non-spatially resolved fashion.

In one embodiment of the invention, the spatially distributed light emitted by a luminophore includes the case of uniform spatial distribution of the light.

In one aspect of the invention, the luminophore is a fluorophore which redistributes through a non-homogenous excitation light field, resulting in a change in the intensity of the light emitted from the luminophore as a result of the change in the amount of excitation light intensity at different points in the field.

In one embodiment of the invention, the recording of the spatially distributed light could be made at a single point in time after the application of the influence. In another embodiment, the recording could be made at two points in time, one point being before, and the other point being after the application of the influence. The result or variation is determined from the change in fluorescence compared to the fluorescence measured prior to the influence or modulation. In another embodiment of the invention, the recording could be performed at a series of points in time, in which the application of the influence occurs at some time after the first time point in the series of recordings, the recording being performed, e.g.. with a predetermined time spacing of from 0.1 seconds to 1 hour, preferably from 1 to 60 seconds, more preferably from 1 to 30 seconds, in particular from 1 to 10 seconds. over a time span of from 1 second to 12 hours, such as from 10 seconds to 12 hours, e.g.. from 10 seconds to one hour, such as from 60 seconds to 30 minutes or 20 minutes. The result or variation is determined from the change in fluorescence over time. The result or variation could also be determined as a change in the spatial distribution of the fluorescence over time.

In one embodiment the recording comprises a time series of total luminescence of the cells of one or several of the spatial limitations. In one embodiment the signal from all of the spatial limitations, one at a time, is measured by a recording being made in the individual spatial limitations by means of an apparatus to sequentially position each one of the limitations in the field of view of the detector and repeating the positioning and measurement process until all of the spatial limitations have been measured. The detector may be a photomultiplier tube. In a preferred embodiment of the present invention more than one spatial limitation is measured simultaneously. This may be done by means of a one- or two-dimensional array detector, whereby the multiple spatial limitations are imaged onto the array detector such that discrete subsets of the detecting units (pixels) in the array detector measure the signal from one and only one of the multiple spatial limitations, the signal from any one spatial limitation being the combined signal from those pixels that receive the image from one of the spatial limitations. This

array detector may be a linear diode array, a video camera (according to any present or future standards and definitions of image acquisition and transmission) or a charge transfer device such as a charge-coupled device (CCD). In one embodiment the recording of signal requires illumination of the multiple spatial limitations to excite the luminophores so that they emit light. In one embodiment all of the spatial limitations are simultaneously illuminated during the measurement. In another embodiment the spatial limitations are singly illuminated only during the time in which they are being measured. In a preferred embodiment the illumination is provided by a laser that is scanned in a raster fashion over some or all of the spatial limitations being measured. The scanning may take place at a rate that is substantially faster than the measurement process such that the illumination appears to the measurement process to be continuous in time and spatially uniform over the region being measured.

The recording of spatially distributed luminescence emitted from the luminophore is performed by an apparatus for measuring the distribution of fluorescence in the cells, and thereby any change in the distribution of fluorescence in the cells, which includes at a minimum the following component parts: (a) a light source, (b) a method for selecting the wavelength(s) of light from the source which will excite the luminescence of the luminophore, (c) a device which can rapidly block or pass the excitation light into the rest of the system, (d) a series of optical elements for conveying the excitation light to the specimen, collecting the emitted fluorescence in a spatially resolved fashion, and forming an image from this fluorescence emission (or another type of intensity map relevant to the method of detection and measurement), (e) a bench or stand which holds the container of the cells being measured in a predetermined geometry with respect to the series of optical elements, (f) a detector to record the spatially resolved fluorescence in the form of an image, (g) a computer or electronic system and associated software to acquire and store the recorded images, and to compute the degree of redistribution from the recorded images.

In a preferred embodiment of the invention the apparatus system is automated. In one embodiment the components in d and e mentioned above comprise a fluorescence microscope. In one embodiment the component in f mentioned above is a CCD camera. In one embodiment the component in f mentioned above is an array of photomultiplier tubes/devices.

In one embodiment the image is formed and recorded by an optical scanning system.

In one embodiment the optical scanning system is used to illuminate the bottom of a plate of microtiter type so that a time-resolved recording of changes in luminescence or fluorescence can be made from all spatial limitations simultaneously.

In a preferred embodiment the actual luminescence or fluorescence measurements are made in a FLIPR™ instrument, commercially available from Molecular Devices, Inc.

In one embodiment of the invention the actual fluorescence measurements are made in a standard type of fluorometer for plates of microtiter type (fluorescence plate reader).

In one embodiment a liquid addition system is used to add a known or unknown compound to any or all of the cells in the cell holder at a time determined in advance. Preferably, the liquid addition system is under the control of the computer or electronic system. Such an automated system can be used for a screening program due to its ability to generate results from a larger number of test compounds than a human operator could generate using the apparatus in a manual fashion.

The methods whereby the detector layer of cells are physically contacted by the compounds can also be of another conceptual type where the compounds are delivered to the cells through a porous membrane by convection/diffusion or by directly contacting compounds attached to an inorganic or organic support (such as glass, plastic or the plasma membrane of intact living cells) with the cells. These methods are thoroughly described in "Method and apparatus for high density format screening for bioactive molecules" the contents of which were part of the priority application, and which, as WO99/35496 has been published during the priority year, are hereby incorporated herein by reference, but are also outlined in the following paragraphs.

In one aspect of the present invention where the detector layer of cells is a continuous or discontinuous sheet of cells without any separation into test units or wells. The compounds are printed onto a nonabsorbent sheet of porous material as a solution in solvent and allowed to dry. This printed sheet of compounds then defines the test pattern for the experiment as it is brought down in close proximity to or in direct contact with the underlying detector layer of cells. The compounds, now dissolved by the fluid layer on

the cells, is brought in contact with the cells through the pores of the membrane by convection. The porous membrane onto which the compounds are printed is optically clear and preferably composed as stated in"Method and apparatus for high density format screening for bioactive molecules" the contents of which were part of the priority application, and which, as WO99/35496 has been published during the priority year, are hereby incorporated herein by reference. In another embodiment of this aspect of the present invention the detector layer of cells is a continuous or discontinuous sheet of cells, without any separation into test units or wells, growing on a porous and optically clear membrane preferably of the types mentioned above. The porous membrane may allow the cells to send cellular processes through the pores of the membrane. The compounds are printed onto an optically clear substratum such as glass, plastic or quartz as solutions in solvent and allowed to dry. At the time of the experiment the cell sheet on the membrane, surrounded by a thin film of fluid, is layered ontop of the printed compound pattern. The compounds then dissolve and contact the cells via diffusion and convection. The compounds may be made using combinatorial chemistry techniques, and may be peptides. The compounds may be covalently attached to the optically clear substratum or porous membrane. The compounds may also be proteins, polypeptides or peptides secreted by or attached to the plasma membrane of genetically modified cells growing as a continuous or discontinuous sheet on a flat optically clear surface or an optically clear porous membrane.

The recording of the variation or result with respect to light emitted from the luminophore is performed by recording the spatially distributed light as one or more digital images, and the processing of the recorded variation to reduce it to one or more numbers representative of the degree of redistribution comprises a digital image processing procedure or combination of digital image processing procedures. The quantitative information which is indicative of the degree of the cellular response to the influence or the result of the influence on the intracellular pathway is extracted from the recording or recordings according to a predetermined calibration based on responses or results, recorded in the same manner, to known degrees of a relevant specific influence. This calibration procedure is developed according to principles described below (Developing an Image-based Assay Technique). Specific descriptions of the procedures for particular assays are given in the examples.

While the stepwise procedure necessary to reduce the image or images to the value representative of the response caused by the influence is particular to each assay, the individual steps are generally well-known methods of image processing. Some examples of the individual steps are point operations such as subtraction, ratioing, and thresholding, digital filtering methods such as smoothing, sharpening, and edge detection, spatial frequency methods such as Fourier filtering, image cross-correlation and image autocorrelation, object finding and classification (blob analysis), and colour space manipulations for visualisation. In addition to the algorithmic procedures, heuristic methods such as neural networks may also be used. In a preferred embodiment of the invention, a dose-response relationship is established based on quantification of the responses caused by a particular influence, representative of the underlying intracellular signalling process, using the methods described above and in the examples. The dose-response relationship for the particular influence is then compared to the dose-response relationship obtained by performing the same assay in an instrument which allows parallel monitoring of all wells in a microtiter plate such as a FLIPR™ or an ordinary fluorescence plate reader for microtiter plates. If a good correlation between the dose-response relationships obtained from the two different measurement systems is obtained, it can be said that the parallel measurement mode has been validated (see examples 8, 9, 10 and 11). This implies that it can be used as the primary basis for a screening assay with the potential benefit of screening a significantly higher number of substances per unit of time for their influence on the response. For example, if the single experiment performed on the microscope can be run in at least 96 experimental chambers simultaneously the throughput for the person who is running the experiments increases by a factor of 96.

Imaging plate readers integrate the signal from each well into a single value per time point. Thus the data resulting from a single "run" of the instrument is a set of time series of single values, one for each well, with the injection of the test compound taking place at a known point in the time series. The primary advantage of this type of instrumentation is that it greatly increases the number of samples that can be processed in a given amount of time (the throughput). This is of great advantage when using the assay in a screening program for new pharmaceutical lead compounds.

The first step in the data analysis is to normalise the results from each well so that they can be compared with each other or with previously analysed known compounds. This

always begins with correcting the signal by subtracting the instrument bias from all data points on a well-by-well basis. From this point, either of two techniques can be followed depending on the design of the assay:

Procedure 1: The average of the signal prior to the addition of the test compound is subtracted from all data points on a well-by-well basis.

Procedure 2: The data are corrected for any known background by subtracting the background value from all data points on a well-by-well basis. The resulting background-corrected data are normalised by dividing each data set by the average of the data values prior to the injection of the test compound on a well-by-well basis.

The corrected or normalised time series data sets are then further reduced by a technique that converts the time series to a single value. There are at least three such approaches:

For transient responses, the maximum deviation from the baseline is determined. This is also known as the "peak height" technique.

Alternatively, the signal is integrated over time between pre-defined limits. If the data were treated according to Procedure 2 above, then the offset is subtracted such that the integral of a non-response is zero within the limit of measurement error. This is also known as the "peak area" technique. If the response is a cumulative one, e.g., an exponential change to a new level, the result is taken as the either the difference or the ratio between the signal after a predetermined time and the signal prior to the addition of the test compound.

All of the above procedures reduce the data for a given well to one or more single values. For screening purposes, these values will be searched for those that are greater than a certain statistically determined cut-off value. For characterisation, the values represent a quantitative response, and are further treated in sets by techniques such as dose-response curve fitting.

In another embodiment of the invention, the measurement of redistribution is accomplished indirectly by taking advantage of the fact that in order for redistribution to occur, the probe will experience some change in its freedom, or restriction, of movement within the intracellular milieu. The degree of translocation will correlate with the amount of freely mobile luminophore in the cytoplasm. At a point in time after the test compound

has begun to have any influence it may have, the amount or fraction of restricted luminophore can be measured by disrupting or permeabilising the plasma membrane of the cells and allowing the freely mobile luminophore to diffuse away. If the detection volume of the detector is limited to the region immediately surrounding the cells, and the overall volume into which the freely mobile luminophore can diffuse is much larger, then the freely mobile luminophore essentially disappears from the detector's view and its signal is not recorded.

In one aspect of the invention, the above mentioned measurement of redistribution is made on cells with permanently permeabilised plasma membranes immersed in a solution mimicking the cytoplasmic environment. In this way the influence of compounds that can normally not enter the cytoplasm of cells can be tested.

The nucleic acid constructs used in the present invention encode in their nucleic acid sequences fusion polypeptides comprising a biologically active polypeptide that is a component of an intracellular signalling pathway, or a part thereof, and a GFP, preferably an F64L mutant of GFP, N- or C-terminally fused, optionally via a peptide linker, to the biologically active polypeptide or part thereof. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a protein kinase or a phosphatase. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a transcription factor or a part thereof which changes cellular localisation upon activation.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a protein, or a part thereof, which is associated with the cytoskeletal network and which changes cellular localisation upon activation. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a protein kinase or a part thereof which changes cellular localisation upon activation. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a serine/threonine protein kinase or a part thereof capable of changing intracellular localisation upon activation. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a tyrosine protein kinase or a part thereof capable of changing intracellular localisation upon activation. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a phospholipid-

dependent serine/threonine protein kinase or a part thereof capable of changing intracellular localisation upon activation.

In a specific embodiment the constructs listed in table 1 are used in a method for extracting quantitative information relating to an influence on a cellular response in mechanically intact or permeablised living cells, the method comprising recording variation in spatially distributed fluorescence emitted from the fluorophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence, as a change fluorescence intensity preferably measured by an instrument designed for the measurement of changes in fluorescence intensity.

Table 1 The fusion constructs of the invention by the names used herein as well as by reference to relevant SEQ ID NOs of sequences of DNA encoding the construct and full amino acid sequences.

Fusion construct	DNA sequence SEQ ID NO:	Protein Sequence SEQ ID NO:
PKAcat - F64LS65TGFP	1	2
PKCα- F64L-S65TGFP	3	4
EGFP - Erk1	5	6
EGFP - SMAD2	7	8
SMAD2 - EGFP	9	10
EGFP - VASP	11	12
EGFP - NFχβ	13	14
NFχβ - EGFP	15	16
EGFP - PKCβ1	17	18

As illustrated in examples 8, 9 and 11, the redistribution of PKA, and PKC can readily be detected as a variation in fluorescence intensity, as measured e.g. in the FLIPR™ instrument.

In one embodiment any new luminophore determined to redistribute in response to an influence in a pattern similar to the pattern observed in the microscope for PKA or PKC (see examples 1, 2, 8 and 11), that is from an aggregated form to a dispersed form or from a dispersed form to an aggregated form of the luminophore as the redistribution takes place, can be predicted to be detectable as a variation in light intensity as measured, for example in the FLIPR™ instrument.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a cAMP-dependent protein kinase or a part thereof capable of changing cellular localisation upon activation. In a preferred embodiment the biologically active polypeptide encoded by the nucleic acid construct is a PKAc-F64L-S65T-GFP fusion. In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a cGMP-dependent protein kinase or a part thereof capable of changing cellular localisation upon activation.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a calmodulin-dependent serine/threonine protein kinase or a part thereof capable of changing cellular localisation upon activation.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a mitogen-activated serine/threonine protein kinase or a part thereof capable of changing cellular localisation upon activation. In preferred embodiments the biologically active polypeptide encoded by the nucleic acid constructs are an ERK1-F64L-S65T-GFP fusion or an EGFP-ERK1 fusion.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a cyclin-dependent serine/threonine protein kinase or a part thereof capable of changing cellular localisation upon activation.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct is a protein phosphatase or a part thereof capable of changing cellular localisation upon activation.

In one preferred embodiment of the invention the nucleic acid constructs may be DNA constructs.

In one embodiment the biologically active polypeptide encoded by the nucleic acid construct. In one embodiment the gene encoding GFP in the nucleic acid construct is derived from Aequorea victoria. In a preferred embodiment the gene encoding GFP in the nucleic acid construct is EGFP or a GFP variant selected from F64L-GFP, F64L-Y66H-GFP and F64L-S65T-GFP.

In preferred embodiments of the invention the DNA constructs which can be identified by any of the DNA sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and 17 or are variants of these sequences capable of encoding the same fusion polypeptide or a fusion polypeptide which is biologically equivalent thereto, e.g. an isoform, or a splice variant or a homologue from another species.

The present invention describes a method that may be used to establish a screening program for the identification of biologically active substances that directly or indirectly affects intracellular signalling pathways and because of this property are potentially useful as medicaments. Based on measurements in living cells of the redistribution of spatially resolved luminescence from luminophores which undergo a change in distribution upon activation or deactivation of an intracellular signalling pathway the result of the individual measurement of each substance being screened indicates its potential biological activity.

In one embodiment of the invention the screening program is used for the identification of a biologically toxic substance as defined herein that exerts its toxic effect by interfering with an intracellular signalling pathway. Based on measurements in living cells of the redistribution of spatially resolved luminescence from luminophores which undergo a change in distribution upon activation or deactivation of an intracellular signalling pathway the result of the individual measurement of each substance being screened indicates its potential biologically toxic activity. In one embodiment of a screening program a compound that modulates a component of an intracellular pathway as defined herein, can be found and the therapeutic amount of the compound estimated by a method according to the method of the invention. In a preferred embodiment the present invention leads to the discovery of a new way of treating a condition or disease related to the intracellular function of a biologically active polypeptide comprising administration to a patient suffering from said condition or disease of an effective amount of a compound which has been discovered by any method according to the invention. In another preferred embodiment of the invention a method is established for identification of a new drug target or several new drug targets among the group of biologically active polypeptides which are components of intracellular signalling pathways.

In another embodiment of the invention an individual treatment regimen is established for the selective treatment of a selected patient suffering from an ailment where the available medicaments used for treatment of the ailment are tested on a relevant primary cell or cells obtained from said patient from one or several tissues, using a method comprising transfecting the cell or cells with at least one DNA sequence encoding a fluorescent probe according to the invention, transferring the transfected cell or cells back the said patient, or culturing the cell or cells under conditions permitting the expression of said probes and exposing it to an array of the available medicaments, then comparing changes in fluorescence patterns or redistribution patterns of the fluorescent probes in the intact living cells to detect the cellular response to the specific medicaments (obtaining a cellular action profile), then selecting one or more medicament or medicaments based on the desired activity and acceptable level of side effects and administering an effective amount of these medicaments to the selected patient.

The present invention describes a method that may be used to establish a screening program for back-tracking signal transduction pathways as defined herein. In one embodiment the screening program is used to establish more precisely at which level one or several compounds affect a specific signal transduction pathway by successively or in parallel testing the influence of the compound or compounds on the redistribution of spatially resolved luminescence from several of the luminophores which undergo a change in distribution upon activation or deactivation of the intracellular signalling pathway under study.

In general, a probe, i.e. a “GeneX”-GFP fusion or a GFP-“GeneX” fusion, is constructed using PCR with “GeneX”-specific primers followed by a cloning step to fuse “GeneX” in frame with GFP. The fusion may contain a short vector derived sequence between “GeneX” and GFP (e.g. part of a multiple cloning site region in the plasmid) resulting in a peptide linker between “GeneX” and GFP in the resulting fusion protein.

Some of the steps involved in the development of a probe include the following:
Identify the sequence of the gene. This is most readily done by searching a depository of genetic information, e.g. the GenBank Sequence Database. which is widely available and routinely used by molecular biologists. In the specific examples below the GenBank Accession number of the gene in question is provided.

Design the gene-specific primers. Inspection of the sequence of the gene allows design of gene-specific primers to be used in a PCR reaction..Typically, the top-strand primer encompasses the ATG start codon of the gene and the following ca. 20 nucleotides, while the bottom-strand primer encompasses the stop codon and the ca. 20 preceding nucleotides, if the gene is to be fused behind GFP, i.e. a GFP-"GeneX" fusion. If the gene is to be fused in front of GFP, i.e. a "GeneX"-GFP fusion, a stop codon must be avoided. Optionally, the full-length sequence of GeneX may not be used in the fusion, but merely the part that localizes and redistributes like GeneX in response to a signal. In addition to gene-specific sequences, the primers contain at least one recognition sequence for a restriction enzyme, to allow subsequent cloning of the PCR product. The sites are chosen so that they are unique in the PCR product and compatible with sites in the cloning vector. Furthermore, it may be necessary to include an exact number of nucleotides between the restriction enzyme site and the gene-specific sequence in order to establish the correct reading frame of the fusion gene and/or a translation initiation consensus sequence. Lastly, the primers always contain a few nucleotides in front of the restriction enzyme site to allow efficient digestion with the enzyme.

Identify a source of the gene to be amplified. In order for a PCR reaction to produce a product with gene-specific primers, the gene-sequence must initially be present in the reaction, e.g. in the form of cDNA. Information in GenBank or the scientific literature will usually indicate in which tissue(s) the gene is expressed, and cDNA libraries from a great variety of tissues or cell types from various species are commercially available, e.g. from Clontech (Palo Alto), Stratagene (La Jolla) and Invitrogen (San Diego). Many genes are also available in cloned form from The American Type Tissue Collection (Virginia).

Optimise the PCR reaction. Several factors are known to influence the efficiency and specificity of a PCR reaction, including the annealing temperature of the primers, the concentration of ions, notably Mg^{2+} and K^{+}, present in the reaction, as well as pH of the reaction. If the result of a PCR reaction is deemed unsatisfactory, it might be because the parameters mentioned above are not optimal. Various annealing temperatures should be tested, e.g. in a PCR machine with a built-in temperature gradient, available from e.g. Stratagene (La Jolla), and/or various buffer compositions should be tried, e.g. the OptiPrime buffer system from Stratagene (La Jolla).

Clone the PCR product. The vector into which the amplified gene product will be cloned and fused with GFP will already have been taken into consideration when the primers

were designed. When choosing a vector, one should at least consider in which cell types the probe subsequently will be expressed, so that the promoter controlling expression of the probe is compatible with the cells. Most expression vectors also contain one or more selective markers, e.g. conferring resistance to a drug, which is a useful feature when one wants to make stable transfectants. The selective marker should also be compatible with the cells to be used.

The actual cloning of the PCR product should present no difficulty as it typically will be a one-step cloning of a fragment digested with two different restriction enzymes into a vector digested with the same two enzymes. If the cloning proves to be problematic, it may be because the restriction enzymes did not work well with the PCR fragment. In this case one could add longer extensions to the end of the primers to overcome a possible difficulty of digestion close to a fragment end, or one could introduce an intermediate cloning step not based on restriction enzyme digestion. Several companies offer systems for this approach, e.g. Invitrogen (San Diego) and Clontech (Palo Alto).

Once the gene has been cloned and, in the process, fused with the GFP gene, the resulting product, usually a plasmid, should be carefully checked to make sure it is as expected. The most exact test would be to obtain the nucleotide sequence of the fusion-gene.

Once a DNA construct for a probe has been generated, its functionality and usefulness may be evaluated by transfecting it into cells capable of expressing the probe. The fluorescence of the cell is inspected soon after, typically the next day. At this point, two features of cellular fluorescence are noted: the intensity and the sub-cellular localisation.

The intensity should usually be at least as strong as that of unfused GFP in the cells. If it is not, the sequence or quality of the probe-DNA might be faulty, and should be carefully checked.

The sub-cellular localisation is an indication of whether the probe is likely to perform well. If it localises as expected for the gene in question, e.g. is excluded from the nucleus, it can immediately go on to a functional test. If the probe is not localised soon after the transfection procedure, it may be because of overexpression at this point in time, as the cell typically will have taken up very many copies of the plasmid, and localisation will

occur in time, e.g. within a few weeks, as plasmid copy number and expression level decreases. If localisation does not occur after prolonged time, it may be because the fusion to GFP has destroyed a localisation function, e.g. masked a protein sequence essential for interaction with its normal cellular anchor-protein. In this case the opposite fusion might work, e.g. if GeneX-GFP does not work, GFP-GeneX might, as two different parts of GeneX will be affected by the proximity to GFP. If this does not work, the proximity of GFP at either end might be a problem, and it could be attempted to increase the distance by incorporating a longer linker between GeneX and GFP in the DNA construct.

If there is no prior knowledge of localisation, and no localisation is observed, it may be because the probe should not be localised at this point, because such is the nature of the protein fused to GFP. It should then be subjected to a functional test.

In a functional test, the cells expressing the probe are treated with at least one compound known to perturb, usually by activating, the signalling pathway on which the probe is expected to report by redistributing itself within the cell. If the redistribution is as expected, e.g. if prior knowledge tell that it should translocate from location X to location Y, it has passed the first critical test. In this case it can go on to further characterisation and quantification of the response.

If it does not perform as expected, it may be because the cell lacks at least one component of the signalling pathway, e.g. a cell surface receptor, or there is species incompatibility, e.g. if the probe is modelled on sequence information of a human gene product, and the cell is of hamster origin. In both instances one should identify other cell types for the testing process where these potential problems would not apply.

If there is no prior knowledge about the pattern of redistribution, the analysis of the redistribution will have to be done in greater depth to identify what the essential and indicative features are, and when this is clear, it can go on to further characterisation and quantification of the response. If no feature of redistribution can be identified, the problem might be as mentioned above, and the probe should be retested under more optimal cellular conditions. If the probe does not perform under optimal cellular conditions, then it's back to the drawing board.

The process of developing an image-based redistribution assay begins with either the unplanned experimental observation that a redistribution phenomenon can be visualised, or the design of a probe specifically to follow a redistribution phenomenon already known to occur. In either event, the first and best exploratory technique is for a trained scientist or technician to observe the phenomenon. Even with the rapid advances in computing technology, the human eye-brain combination is still the most powerful pattern recognition system known, and requires no advance knowledge of the system in order to detect potentially interesting and useful patterns in raw data. This is especially if those data are presented in the form of images, which are the natural "data type" for human visual processing. Because human visual processing operates most effectively in a relatively narrow frequency range. i.e., we cannot see either very fast or very slow changes in our visual field, it may be necessary to record the data and play it back with either time dilation or time compression.

Some luminescence phenomena cannot be seen directly by the human eye. Examples include polarisation and fluorescence lifetime. However, with suitable filters or detectors, these signals can be recorded as images or sequences of images and displayed to the human in the fashion just described. In this way, patterns can be detected and the same methods can be applied.

Once the redistribution has been determined to be a reproducible phenomenon, one or more data sets are generated for the purpose of developing a procedure for extracting the quantitative information from the data. In parallel, the biological and optical conditions are determined which will give the best quality raw data for the assay. This can become an iterative process: it may be necessary to develop a quantitative procedure in order to assess the effect on the assay of manipulating the assay conditions.

The data sets are examined by a person or persons with knowledge of the biological phenomenon and skill in the application of image processing techniques. The goal of this exercise is to determine or at least propose a method that will reduce the image or sequence of images constituting the record of a "response" to a value corresponding to the degree of the response. Using either interactive image processing software or an image processing toolbox and a programming language, the method is encoded as a procedure or algorithm that takes the image or images as input and generates the

degree of response (in any units) as its output. Some of the criteria for evaluating the validity of a particular procedure are:

Does the degree of the response vary in a biologically significant fashion, i.e., does it show the known or putative dependence on the concentration of the stimulating agent or condition?

Is the degree of response reproducible, i.e., does the same concentration or level of stimulating agent or condition give the same response with an acceptable variance? Is the dynamic range of the response sufficient for the purpose of the assay? If not, can a change in the procedure or one of its parameters improve the dynamic range? Does the procedure exhibit any clear "pathologies", i.e., does it give ridiculous values for the response if there are commonly occurring imperfections in the imaging process? Can these pathologies be eliminated, controlled, or accounted for? Can the procedure deal with the normal variation in the number and/or size of cells in an image?

In some cases the method may be obvious; in others, a number of possible procedures may suggest themselves. Even if one method appears clearly superior to others, optimisation of parameters may be required. The various procedures are applied to the data set and the criteria suggested above are determined, or the single procedure is applied repeatedly with adjustment of the parameter or parameters until the most satisfactory combination of signal. noise, range, etc. are arrived at. This is equivalent to the calibration of any type of single-channel sensor.

The number of ways of extracting a single value from an image are extremely large, and thus an intelligent approach must be taken to the initial step of reducing this number to a small, finite number of possible procedures. This is not to say that the procedure arrived at is necessarily the best procedure - but a global search for the best procedure is simply out of the question due to the sheer number of possibilities involved.

Image-based assays are no different than other assay techniques in that their usefulness is characterised by parameters such as the specificity for the desired component of the sample, the dynamic range, the variance, the sensitivity, the concentration range over which the assay will work, and other such parameters. While it is not necessary to

characterise each and every one of these before using the assay, they represent the only way to compare one assay with another.

The final step is then to see whether there exists a possibility to increase the throughput of the assay to improve its utility as the basis of a screening program. In order to do this, a dose-response relationship is established based on quantification of the responses caused by a particular influence, representative of the underlying intracellular signalling process, using the methods described above and in the examples. The dose-response relationship for the particular influence is then compared to the dose-response relationship obtained by performing the same assay in an instrument which allows parallel monitoring of all wells in a microtiter plate such as a FLIPR™ or an ordinary imaging or fluorescence plate reader for microtiter plates. If a good correlation between the dose-response relationships obtained from the two different measurement systems is obtained, it can be said that the parallel measurement mode has been validated (see examples 8, 9 and 11). This implies that it can be used as the primary basis for a screening program with the potential benefit of screening a significantly higher number of substances for their influence on the response per unit of time.

In the present specification and claims, the term "an influence" covers any influence to which the cellular response comprises a redistribution. Thus, e.g., heating, cooling, pH, high pressure, low pressure, humidifying, or drying are influences on the cellular response on which the resulting redistribution can be quantified, but as mentioned above, perhaps the most important influences are the influences of contacting or incubating the cells with substances which are known or suspected to exert an influence on the cellular response involving a redistribution contribution. In another embodiment of the invention the influence could be substances from a compound drug library.

In the present context, the term “green fluorescent protein” is intended to indicate a protein which, when expressed by a cell, emits fluorescence upon exposure to light of the correct excitation wavelength (cf. [(Chalfie, M. *et al.* (1994) Science 263, 802-805)]). In the following, GFP in which one or more amino acids have been substituted, inserted or deleted is most often termed “modified GFP”. “GFP” as used herein includes wild-type GFP derived from the jelly fish *Aequorea victoria* and modifications of GFP, such as the blue fluorescent variant of GFP disclosed by Heim *et al.* (1994). Proc.Natl.Acad.Sci. 91:26, pp 12501-12504, and other modifications that change the spectral properties of the GFP

fluorescence, or modifications that exhibit increased fluorescence when expressed in cells at a temperature above about 30°C described in PCT/DK96/00051, published as WO 97/11094 on 27 March 1997 and hereby incorporated by reference, and which comprises a fluorescent protein derived from *Aequorea* Green Fluorescent Protein (GFP) or any functional analogue thereof, wherein the amino acid in position 1 upstream from the chromophore has been mutated to provide an increase of fluorescence intensity when the fluorescent protein of the invention is expressed in cells. Preferred GFP variants are F64L-GFP, F64L-Y66H-GFP and F64L-S65T-GFP. An especially preferred variant of GFP for use in all the aspects of this invention is EGFP (DNA encoding EGFP which is a F64L-S65T variant with codons optimized for expression in mammalian cells is available from Clontech, Palo Alto, plasmids containing the EGFP DNA sequence, cf. GenBank Acc. Nos. U55762, U55763).

The term "intracellular signalling pathway" and "signal transduction pathway" are intended to indicate the co-ordinated intracellular processes whereby a living cell transduce an external or internal signal into cellular responses. Said signal transduction will involve an enzymatic reaction said enzymes include but are not limited to protein kinases, GTPases, ATPases, protein phosphatases, phospholipases and cyclic nucleotide phosphodiesterases. The cellular responses include but are not limited to gene transcription, secretion, proliferation, mechanical activity, metabolic activity, cell death.

The term "second messenger" is used to indicate a low molecular weight component involved in the early events of intracellular signal transduction pathways.

The term "luminophore" is used to indicate a chemical substance that has the property of emitting light either inherently or upon stimulation with chemical or physical means. This includes but is not limited to fluorescence, bioluminescence. phosphorescence, and chemiluminescence.

The term "mechanically intact living cell" is used to indicate a cell which is considered living according to standard criteria for that particular type of cell such as maintenance of normal membrane potential, energy metabolism, proliferative capability, and has not experienced any physically invasive treatment designed to introduce external substances into the cell such as microinjection.

In the present context, the term "permeabilised living cell" is used to indicate cells where a pore forming agent such as Streptolysin O or *Staphylococcus Aureus* α-toxin has been applied and thereby incorporated into the plasma membrane in the cells. This creates proteinaceous pores with a defined pore size in the plasma membranes of the exposed cells. Pores could also be made by electroporation, i.e. exposing the cells to high voltage discharges, a procedure that creates small holes in the plasma membrane by coagulating integral membrane proteins. Treatment with a mild detergent such as saponin may accomplish the same thing. Common to all these treatments are that pores are formed only in the plasma membrane without affecting the integrity of cytoplasmic structural elements and organelles. The term living in this context means that the permeabilised cells bathed in a solution mimicking the intracellular milieu still have functional organelles, such as actively respiring mitochondria and endoplasmic reticulum that can take up and release calcium ions, and functional structural elements. The benefit of this method is that substances that normally can not traverse the plasma membrane, but most likely exert their influence intracellularly, can be introduced and their influence studied without cumbersome microinjection of the substances into single cells. Using this method the response to an influence can be recorded from many cells simultaneously.

In the present context, the term "permeabilisation" is intended to indicate the selective disruption of the plasma membrane barrier so that soluble substances freely mobile in the cytosol are lost from the cells. The permeabilisation can be achieved as described above under "permeabilised living cells" or by using other chemical detergents such as Triton X-100 or digitonin in carefully titrated amounts.

The term "physiologically relevant", when applied to an experimentally determined redistribution of an intracellular component, as measured by a change in the luminescence properties or distribution, is used to indicate that said redistribution can be explained in terms of the underlying biological phenomenon which gives rise to the redistribution.

The terms "image processing" and "image analysis" are used to describe a large family of digital data analysis techniques or combination of such techniques which reduce ordered arrays of numbers (images) to quantitative information describing those ordered

arrays of numbers. When said ordered arrays of numbers represent measured values from a physical process, the quantitative information derived is therefore a measure of the physical process.

The term "fluorescent probe" is used to indicate a fluorescent fusion polypeptide comprising a GFP or any functional part thereof which is N- or C-terminally fused to a biologically active polypeptide as defined herein, optionally via a peptide linker consisting of one or more amino acid residues, where the size of the linker peptide in itself is not critical as long as the desired functionality of the fluorescent probe is maintained. A fluorescent probe according to the invention is expressed in a cell and basically mimics the physiological behaviour of the biologically active polypeptide moiety of the fusion polypeptide.

The term "mammalian cell" is intended to indicate any living cell of mammalian origin. The cell may be an established cell line, many of which are available from The American Type Culture Collection (ATCC. Virginia, USA) or a primary cell with a limited life span derived from a mammalian tissue. including tissues derived from a transgenic animal, or a newly established immortal cell line derived from a mammalian tissue including transgenic tissues, or a hybrid cell or cell line derived by fusing different cell types of mammalian origin e.g. hybridoma cell lines. The cells may optionally express one or more non-native gene products. e.g. receptors, enzymes, enzyme substrates, prior to or in addition to the fluorescent probe. Preferred cell lines include but are not limited to those of fibroblast origin, e.g. BHK. CHO, BALB, or of endothelial origin, e.g. HUVEC, BAE (bovine artery endothelial). CPAE (cow pulmonary artery endothelial), HLMVEC (human lung microvascular endothelial cells) or of pancreatic origin, e.g. RIN, INS-1, MIN6, bTC3, aTC6, bTC6, HIT, or of hematopoietic origin, e.g.primary isolated human monocytes, macrophages, neutrophils, basophils, eosinophils and lyphocyte populations, AML-193, HL-60, RBL-1, adipocyte origin, e.g. 3T3-L1, neuronal/neuroendocrine origin. e.g. AtT20, PC12, GH3, muscle origin, e.g. SKMC, A10, C2C12, renal origin, e.g. HEK 293. LLC-PK1.

The term "hybrid polypeptide" is intended to indicate a polypeptide which is a fusion of at least a portion of each of two proteins, in this case at least a portion of the green fluorescent protein, and at least a portion of a catalytic and/or regulatory domain of a protein kinase. Furthermore a hybrid polypeptide is intended to indicate a fusion

polypeptide comprising a GFP or at least a portion of the green fluorescent protein that contains a functional fluorophore, and at least a portion of a biologically active polypeptide. Thus, GFP may be N- or C-terminally tagged to a biologically active polypeptide, optionally via a linker portion or linker peptide consisting of a sequence of one or more amino acids. The hybrid polypeptide or fusion polypeptide may act as a fluorescent probe in intact living cells carrying a DNA sequence encoding the hybrid polypeptide under conditions permitting expression of said hybrid polypeptide.

The term "kinase" is intended to indicate an enzyme that is capable of phosphorylating a cellular component.

The term "protein kinase" is intended to indicate an enzyme that is capable of phosphorylating serine and/or threonine and/or tyrosine in peptides and/or proteins.

The term "phosphatase" is intended to indicate an enzyme that is capable of dephosphorylating phosphoserine and/or phosphothreonine and/or phosphotyrosine in peptides and/or proteins.

The term "cyclic nucleotide phosphodiesterase" is intended to indicate an enzyme that is capable of inactivating the second messengers cAMP and cGMP by hydrolysis of their 3'-ester bond.

In the present context, the term "biologically active polypeptide" is intended to indicate a polypeptide affecting intracellular processes upon activation, such as an enzyme which is active in intracellular processes or a portion thereof comprising a desired amino acid sequence which has a biological function or exerts a biological effect in a cellular system. In the polypeptide one or several amino acids may have been deleted, inserted or replaced to alter its biological function, e.g. by rendering a catalytic site inactive. Preferably, the biologically active polypeptide is selected from the group consisting of proteins taking part in an intracellular signalling pathway, such as enzymes involved in the intracellular phosphorylation and dephosphorylation processes including kinases, protein kinases and phosphorylases as defined herein, but also proteins making up the cytoskeleton play important roles in intracellular signal transduction and are therefore included in the meaning of "biologically active polypeptide" herein. More preferably, the biologically active polypeptide is a protein which according to its state as activated or

non-activated changes localisation within the cell, preferably as an intermediary component in a signal transduction pathway. Included in this preferred group of biologically active polypeptides are cAMP dependent protein kinase A.

The term “a substance having biological activity” is intended to indicate any sample that has a biological function or exerts a biological effect in a cellular system. The sample may be a sample of a biological material such as a sample of a body fluid including blood, plasma, saliva, milk, urine, or a microbial or plant extract, an environmental sample containing pollutants including heavy metals or toxins. or it may be a sample containing a compound or mixture of compounds prepared by organic synthesis or genetic techniques.

The phrase “any change in fluorescence” means any change in absorption properties, such as wavelength and intensity, or any change in spectral properties of the emitted light, such as a change of wavelength, fluorescence lifetime, intensity or polarisation, or any change in the intracellular localisation of the fluorophore. It may thus be localised to a specific cellular component (e.g. organelle, membrane, cytoskeleton, molecular structure) or it may be evenly distributed throughout the cell or parts of the cell.

The term “organism” as used herein indicates any unicellular or multicellular organism preferably originating from the animal kingdom including protozoans, but also organisms that are members of the plant kingdoms, such as algae, fungi. bryophytes, and vascular plants are included in this definition.

The term “nucleic acid” is intended to indicate any type of poly- or oligonucleic acid sequence, such as a DNA sequence, a cDNA sequence, or an RNA sequence.

The term “biologically equivalent” as it relates to proteins is intended to mean that a first protein is equivalent to a second protein if the cellular functions of the two proteins may substitute for each other, e.g. if the two proteins are closely related isoforms encoded by different genes, if they are splicing variants, or allelic variants derived from the same gene, if they perform identical cellular functions in different cell types, or in different species. The term “biologically equivalent” as it relates to DNA is intended to mean that a first DNA sequence encoding a polypeptide is equivalent to a second DNA sequence

encoding a polypeptide if the functional proteins encoded by the two genes are biologically equivalent.

The term "higher throughput" is intended to mean an increased number of experiments per time unit per person performing the actual experiments.

The term "high throughput screening assay" as used herein is intended to mean the process of performing a screening assay with at least 100 individual experiments where compounds are tested for their influence on the redistribution of a luminophore in one working day for one person skilled in the art. In a preferred embodiment the high throughput screening assay involves at least 500 individual experiments such as 750, 1000, 2000, 5000, or even 10.000 individual experiments in one working day for a person skilled in the art.

The phrase "back-tracking of a signal transduction pathway" is intended to indicate a process for defining more precisely at what level a signal transduction pathway is affected, either by the influence of chemical compounds or a disease state in an organism. Consider a specific signal transduction pathway represented by the bioactive polypeptides A - B - C - D, with signal transduction from A towards D. When investigating all components of this signal transduction pathway compounds or disease states that influence the activity or redistribution of only D can be considered to act on C or downstream of C whereas compounds or disease states that influence the activity or redistribution of C and D, but not of A and B can be considered to act downstream of B.

The term "fixed cells" is used to mean cells treated with a cytological fixative such as glutaraldehyde or formaldehyde, treatments that serve to chemically cross-link and stabilise soluble and insoluble proteins within the structure of the cell. Once in this state, such proteins cannot be lost from the structure of the now-dead cell.

In the present context a "screening assay" is intended to mean any measurement protocol, including materials, cells. instruments, chemicals, reagents, detection units, calibration and quantification procedures used to measure a response from mechanically intact or permeabilised living cells relevant to influences on an intracellular pathway.

The term "dose-response relationship" and "screening programme" is in the present context intended to mean a clear correlation between the quantified response of cells in a screening assay to application of an influence, such as a compound, and the concentration of the applied influence. The response to the influence may be both an up-regulation and a down-regulation of the quantified parameter used in the screening assay.

In the present context, the term "physiology" is intended to mean the normal function of biological and biochemical processes inside cells, between cells and in the whole organism or animal.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. CHO cells expressing the PKAc-F64L-S65T-GFP hybrid protein have been treated in HAM's F12 medium with 50 μM forskolin at 37°C. The images of the GFP fluorescence in these cells have been taken at different time intervals after treatment, which were: a) 40 seconds b) 60 seconds c) 70 seconds d) 80 seconds. The fluorescence changes from a punctate to a more even distribution within the (non-nuclear) cytoplasm.

Figure 2. Time-lapse analysis of forskolin induced PKAc-F64L-S65T-GFP redistribution. CHO cells, expressing the PKAc-F64L-S65T-GFP fusion protein were analysed by time-lapse fluorescence microscopy. Fluorescence micrographs were acquired at regular intervals from 2 min before to 8 min after the addition of agonist. The cells were challenged with 1 μM forskolin immediately after the upper left image was acquired (t=0). Frames were collected at the following times: i) 0, ii) 1, iii) 2, iv) 3, v) 4 and vi) 5 minutes. Scale bar 10 μm.

Figure 3. Time-lapse analyses of PKAc-F64L-S65T-GFP redistribution in response to various agonists. The effects of 1 μM forskolin (A), 50 μM forskolin (B), 1mM dbcAMP (C) and 100 μM IBMX (D) (additions indicated by open arrows) on the localisation of the PKAc-F64L-S65T-GFP fusion protein were analysed by time-lapse fluorescence microscopy of CHO/PKAc-F64L-S65T-GFP cells. The effect of addition of 10 μM forskolin (open arrow), followed shortly by repeated washing with buffer (solid arrow), on the localisation of the PKAc-F64L-S65T-GFP fusion protein was analysed in the same

cells (E). In a parallel experiment, the effect of adding 10 μM forskolin and 100 μM IBMX (open arrow) followed by repeated washing with buffer containing 100 μM IBMX (solid arrow) was analysed (F). Removing forskolin caused PKAc-F64L-S65T-GFP fusion protein to return to the cytoplasmic aggregates while this is prevented by the continued presence of IBMX (F). The effect of 100 nM glucagon (Fig 3G, open arrow) on the localisation of the PKAc-F64L-S65T-GFP fusion protein is also shown for BHK/GR, PKAc-F64L-S65T-GFP cells. The effect of 10 μM norepinephrine (H), solid arrow, on the localisation of the PKAc-F64L-S65T-GFP fusion protein was analysed similarly, in transiently transfected CHO, PKAc-F64L-S65T-GFP cells, pretreated with 10 μM forskolin, open arrow, to increase [cAMP]. N.B. in Fig 3H the x-axis counts the image numbers, with 12 seconds between images. The raw data of each experiment consisted of 60 fluorescence micrographs acquired at regular intervals including several images acquired before the addition of buffer or agonist. The charts (A-G) each show a quantification of the response seen through all the 60 images. performed as described in analysis method 2. The change in total area of the highly fluorescent aggregates, relative to the initial area of fluorescent aggregates is plotted as the ordinate in all graphs in Figure 3, versus time for each experiment. Scale bar 10 μm.

Figure 4. Dose-response curve (two experiments) for forskolin-induced redistribution of the PKAc-F64L-S65T-GFP fusion.

Figure 5. Time from initiation of a response to half maximal ($t_{1/2max}$) and maximal (t_{max}) PKAc-F64L-S65T-GFP redistribution. The data was extracted from curves such as that shown in "Figure 2." All $t_{1/2max}$ and t_{max} values are given as mean±SD and are based on a total of 26-30 cells from 2-3 independent experiments for each forskolin concentration. Since the observed redistribution is sustained over time, the t_{max} values were taken as the earliest time point at which complete redistribution is reached. Note that the values do not relate to the degree of redistribution.

Figure 6. Parallel dose-response analyses of forskolin induced cAMP elevation and PKAc-F64L-S65T-GFP redistribution. The effects of buffer or 5 increasing concentrations of forskolin on the localisation of the PKAc-F64L-S65T-GFP fusion protein in CHO/PKAc-F64L-S65T-GFP cells, grown in a 96 well plate, were analysed as described above. Computing the ratio of the SD's of fluorescence micrographs taken of the same field of cells, prior to and 30 min after the addition of forskolin, gave a reproducible measure of

PKAc-F64L-S65T-GFP redistribution. The graph shows the individual 48 measurements and a trace of their mean±s.e.m at each forskolin concentration. For comparison, the effects of buffer or 8 increasing concentrations of forskolin on $[cAMP]_i$ was analysed by a scintillation proximity assay of cells grown under the same conditions. The graph shows a trace of the mean ± s.e.m of 4 experiments expressed in arbitrary units.

Figure 7. BHK cells stably transfected with the human muscarinic (hM1) receptor and the PKCα-F64L-S65T-GFP fusion. Carbachol (100 μM added at 1.0 second) induced a transient redistribution of PKCα-F64L-S65T-GFP from the cytoplasm to the plasma membrane. Images were taken at the following times: a) 1 second before carbachol addition, b) 8.8 seconds after addition and c) 52.8 seconds after addition.

Figure 8. BHK cells stably transfected with the hM1 receptor and PKCα-F64L-S65T-GFP fusion were treated with carbachol (1 μM, 10 μM, 100 μM). In single cells intracellular $[Ca^{2+}]$ was monitored simultaneously with the redistribution of PKCα-F64L-S65T-GFP. Dashed line indicates the addition times of carbachol. The top panel shows changes in the intracellular Ca^{2+} concentration of individual cells with time for each treatment. The middle panel shows changes in the average cytoplasmic GFP fluorescence for individual cells against time for each treatment. The bottom panel shows changes in the fluorescence of the periphery of single cells, within regions that specifically include the circumferential edge of a cell as seen in normal projection, the best regions for monitoring changes in the fluorescence intensity of the plasma membrane.

Figure 9. The hERK1-F64L-S65T-GFP fusion expressed in HEK293 cells treated with 100 μM of the MEK1 inhibitor PD98059 in HAM F-12 (without serum) for 30 minutes at 37 °C. The nuclei empty of fluorescence during this treatment. The same cells as in (a) following treatment with 10 % foetal calf serum for 15 minutes at 37 °C.
Time profiles for the redistribution of GFP fluorescence in HEK293 cells following treatment with various concentrations of EGF in Hepes buffer (HAM F-12 replaced with Hepes buffer directly before the experiment). Redistribution of fluorescence is expressed as the change in the ratio value between areas in nucleus and cytoplasm of single cells. Each time profile is the mean for the changes seen in six single cells.
Bar chart for the end-point measurements, 600 seconds after start of EGF treatments, of fluorescence change (nucleus:cytoplasm) following various concentrations of EGF.

Figure 10. The SMAD2-EGFP fusion expressed in HEK293 cells starved of serum overnight in HAM F-12. HAM F-12 was then replaced with Hepes buffer pH 7.2 immediately before the experiment. Scale bar is 10 μm.
HEK 293 cells expressing the SMAD2-EGFP fusion were treated with various concentration of TGF-beta as indicated, and the redistribution of fluorescence monitored against time. The time profile plots represent increases in fluorescence within the nucleus, normalised to starting values in each cell measured. Each trace is the time profile for a single cell nucleus.
A bar chart representing the end-point change in fluorescence within nuclei (after 850 seconds of treatment) for different concentrations of TGF-beta. Each bar is the value for a single nucleus in each treatment.

Figure 11. The VASP-F64L-S65T-GFP fusion in CHO cells stably transfected with the human insulin receptor. The cells were starved for two hours in HAM F-12 without serum, then treated with 10% foetal calf serum. The image shows the resulting redistribution of fluorescence after 15 minutes of treatment. GFP fluorescence becomes localised in structures identified as focal adhesions along the length of actin stress fibres.

Figure 12. Dose-response relationship for the translocation of PKCα-GFP in BHKhM1 cells stimulated with the muscarininc agonist carbamylcholine using a FLIPR™ to do the actual experiments.

Figure 13. Dose-response relationship for the translocation of PKAc-GFP in CHO/PKAc-F64L-S65T-GFP cells stimulated with forskolin using a FLIPR™ to do the actual experiments.

Figure 14. CHO cells stably expressing the human insulin receptor and mouse cPKA labeled with S65T-GFP were more thoroughly investigated in the FLIPR™ instrument. A forskolin (a substance that increases Adenylate cyclase production of cAMP in the cells) dose-response was created where six separate wells were imaged over time for each concentration. The changes in fluorescence were calculated as AUC (area under the curve) for 9 min of stimulation.
Conclusion: Redistribution of mouse cPKA - BioST can be detected in the FLIPR™ despite the fact that the whole wells, containing around 50-100 000 cells, are illuminated and imaged simultaneously with a spatial resolution that is far from capable of resolving

single cells or subcellular events. The method can be used as a real time measurement of cAMP levels in the cells and as a screening assay to measure effects of ligands to G-protein coupled receptors linked to Gi and Gq type G-proteins.

Figure 15. Dose-response relationship for the disappearance of fluorescence from permeabilised CHO/PKAc-F64L-S65T-GFP when previously exposed to different doses of forskolin.

Figure 16. CHO cells stably expressing the human insulin receptor and human PKC beta 1 labeled with EGFP were investigated in the microscope. A dose-response was created where a set of cells were imaged over time for each concentration. The changes in fluorescence were calculated as AUC for 4 min of stimulation. From the images the following data were extracted:

Whole image : Just analysing the change in intensity in the whole images taking both cells and background.

Single cell: 5 separate cells were analysed after background compensation. The analysis was made on the entire cell.

Cytoplasm: The same 5 cells as above were analysed after background compensation. the analysis was made on a small region in the cytoplasm close to the nucleus.

Conclusion: Redistribution of human PKC beta 1 – EGFP can only be detected if a subregion of each cell is analysed. The event is clearly visible when the image series is viewed as a movie but if the whole image change in fluorescence or the change in fluorescence in entire cells are analysed the redistribution cannot be detected.

Figure 17. CHO cells stably expressing the human insulin receptor and human PKC beta 1 labeled with EGFP were investigated in the FLIPR™. A dose-response was created where six separate wells were imaged over time for each concentration. The changes in fluorescence were calculated as AUC for 5 min of stimulation.

Conclusion: Redistribution of human PKC beta 1 – EGFP can be detected in the FLIPR™ despite the fact that the whole wells, containing around 50-100 000 cells, are illuminated and imaged by a detector that has a resolution far below that needed to resolve single cells or subcellular structures. This phenomenon can clearly not be predicted from the microscope data in Figure 16.

Figure 18 CHO cells stably expressing the insulin receptor and a human NFkB – GFP protein hybrid were stimulated with different concentrations of IL-1 for 1 h, then washed with a hypoosmotic buffer (TRIS-base 10mM, MgCl2 2mM, PMSF(Phenyl methyl sulfonyl fluoride) 0.5mM, pH 7.4) and placed on the microscope. A sequence of images were acquired during the addition of 0.05% Triton X-100 and subsequent gentle mixing after a short incubation period. The treatment causes the cell membranes to rupture leaving the fraction of NFkB-GFP that has translocated to the nucleus behind whereas the cytoplasmic amount of the probe leaves the cells more quickly and immediately becomes infinitely diluted in the surrounding medium (out of focus - this part of the total fluorescence from the probe is thereby lost). At a defined time point before and after this treatment a total intensity value for the whole image was extracted. To normalize each experiment, the after value was divided by the before value. meaning that a higher ratio was found in cells where more NFkB had translocated to the nucleus and thereby contributed to the total fluorescence after permeabilisation.

Conclusion: the present protocol is a good example of the possibility of revealing translocation of a fluorescent probe from the cytosol to the nucleus or translocation from the nucleus to the cytosol.

EXAMPLES

EXAMPLE 1 Construction, testing and implementation of an assay for cAMP based on PKA activation.

Useful for monitoring the activity of signalling pathways that lead to altered concentrations of cAMP, e.g. activation of G-protein coupled receptors which couple to G-proteins of the G_S or G_I class.

The catalytic subunit of the murine cAMP dependent protein kinase (PKAc) was fused C-terminally to a F64L-S65T derivative of GFP. The resulting fusion (PKAc-F64L-S65T-GFP) was used for monitoring *in vivo* the translocation and thereby the activation of PKA.

To construct the PKAc-F64L-S65T-GFP fusion, convenient restriction endonuclease sites were introduced into the cDNAs encoding murine PKAc (Gen Bank Accession number: M12303) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) by polymerase chain reaction (PCR). The PCR reactions were performed according to standard protocols with the following primers:

5'PKAc:

TTggACACAAgCTTTggACACCCTCAggATATgggCAACgCCgCCgCCgCCAAg,

3'PKAc:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCAAACTCAgTAAACTCCTTgCCACAC

5'GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTTC

3'GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT.

The PKAc amplification product was then digested with HindIII+AscI and the F64L-S65T-GFP product with AscI+XhoI. The two digested PCR products were subsequently ligated with a HindIII+XhoI digested plasmid (pZeoSV® mammalian expression vector, Invitrogen, San Diego, CA, USA). The resulting fusion construct (SEQ ID NO:1 and 2) was under control of the SV40 promoter.

Transfection and cell culture conditions:

Chinese hamster ovary cells (CHO), were transfected with the plasmid containing the PKAc-F64L-S65T-GFP fusion using the calcium phosphate precipitate method in HEPES-buffered saline (Sambrook *et al.*, 1989). Stable transfectants were selected using 1000 μg Zeocin/ml (Invitrogen) in the growth medium (DMEM with 1000 mg glucose/l, 10 % fetal bovine serum (FBS), 100 μg penicillin-streptomycin mixture ml^{-1}, 2 mM L-glutamine purchased from Life Technologies Inc., Gaithersburg, MD, USA). Untransfected CHO cells were used as the control. To assess the effect of glucagon on fusion protein translocation, the PKAc-F64L-S65T-GFP fusion was stably expressed in baby hamster kidney cells overexpressing the human glucagon receptor (BHK/GR cells). Untransfected BHK/GR cells were used as the control. Expression of GR was maintained with 500 μg G418/ml (*Neo* marker) andPKAc-F64L-S65T-GFP was maintained with 500 μg Zeocin/ml (*Sh ble* marker). CHO cells were also simultaneously co-transfected with vectors containing the PKAc-F64L-S65T-GFP fusion and the human α2a adrenoceptor (hARa2a).

For fluorescence microscopy, cells were allowed to adhere to Lab-Tek chambered coverglasses (Nalge Nunc Int., Naperville, IL, USA) for at least 24 hours and cultured to about 80% confluence. Prior to experiments, the cells were cultured over night without selection pressure in HAM F-12 medium with glutamax (Life Technologies), 100 μg penicillin-streptomycin mixture ml^{-1} and 0.3 % FBS. This medium has low autofluorescence enabling fluorescence microscopy of cells straight from the incubator.

Monitoring activity of PKA activity in real time:

Image aquisition of live cells were gathered using a Zeiss Axiovert 135M fluorescence microscope fitted with a Fluar 40X. NA: 1.3 oil immersion objective and coupled to a Photometrics CH250 charged coupled device (CCD) camera. The cells were illuminated with a 100 W HBO arc lamp. In the light path was a 470±20 nm excitation filter, a 510 nm dichroic mirror and a 515±15 nm emission filter for minimal image background. The cells were maintained at 37°C with a custom built stage heater.

Images were processed and analysed in the following manner:

Method 1: Stepwise procedure for quantitation of translocation of PKA:

The image was corrected for dark current by performing a pixel-by-pixel subtraction of a dark image (an image taken under the same conditions as the actual image, except the camera shutter is not allowed to open).

The image was corrected for non-uniformity of the illumination by performing a pixel-by-pixel ratio with a flat field correction image (an image taken under the same conditions as the actual image of a uniformly fluorescent specimen).

The image histogram, i.e., the frequency of occurrence of each intensity value in the image, was calculated.

A smoothed, second derivative of the histogram was calculated and the second zero is determined. This zero corresponds to the inflection point of the histogram on the high side of the main peak representing the bulk of the image pixel values.

The value determined in step 4 was subtracted from the image. All negative values were discarded.

The variance (square of the standard deviation) of the remaining pixel values was determined. This value represents the "response" for that image.

Scintillation proximity assay (SPA) for independent quantitation of cAMP.

Method 2: Alternative method for quantitation of PKA redistribution:

The fluorescent aggregates are segmented from each image using an automatically found threshold based on the maximisation of the information measure between the object and background. The *a priori* entropy of the image histogram is used as the information measure.

The area of each image occupied by the aggregates is calculated by counting pixels in the segmented areas.

The value obtained in step 2 for each image in a series, or treatment pair, is normalised to the value found for the first (unstimulated) image collected. A value of zero (0) indicates no redistribution of fluorescence from the starting condition. A value of one (1) by this method equals full redistribution.

Cells were cultured in HAM F-12 medium as described above, but in 96-well plates. The medium was exchanged with Ca^{2+}-HEPES buffer including 100 μM IBMX and the cells were stimulated with different concentrations of forskolin for 10 min. Reactions were stopped with addition of NaOH to 0.14 M and the amount of cAMP produced was measured with the cAMP-SPA kit, RPA538 (Amersham) as described by the manufacturer.

Manipulating intracellular levels of cAMP to test the PKAc-F64L-S65T-GFP fusion.

The following compounds were used to vary cAMP levels: Forskolin, an activator of adenylate cyclase; dbcAMP, a membrane permeable cAMP analog which is not degraded by phosphodiesterase; IBMX, an inhibitor of phosphodiesterase.
CHO cells stably expressing the PKAc-F64L-S65T-GFP, showed a dramatic translocation of the fusion protein from a punctate distribution to an even distribution throughout the cytoplasm following stimulation with 1 μM forskolin (n=3), 10 μM forskolin (n=4) and 50 μM forskolin (n=4) (Fig 1), or dbcAMP at 1mM (n=6).
Fig. 2 shows the progression of response in time following treatment with 1 μM forskolin.
Fig. 3 gives a comparison of the average temporal profiles of fusion protein redistribution and a measure of the extent of each response to the three forskolin concentrations (Fig. 3A, E, B), and to 1 mM dbcAMP (fig 3C) which caused a similar but slower response, and to addition of 100 μM IBMX (n=4, Fig. 3D) which also caused a slow response, even in the absence of adenylate cyclase stimulation. Addition of buffer (n=2) had no effect (data not shown).
As a control for the behaviour of the fusion protein, F64L-S65T-GFP alone was expressed in CHO cells and these were also given 50 μM forskolin (n=5); the uniform diffuse distribution characteristic of GFP in these cells was unaffected by such treatment (data not shown).
The forskolin-induced translocation of PKAc-F64L-S65T-GFP showed a dose-response relationship (Fig 4 and 6), see quantitative procedures above.

Reversibility of PKAc-F64L-S65T-GFP translocation.
The release of the PKAc probe from its cytoplasmic anchoring hotspots was reversible. Washing the cells repeatedly (5-8 times) with buffer after 10μM forskolin treatment completely restored the punctate pattern within 2-5 min (n=2. Fig. 3E). In fact the fusion protein returned to a pattern of fluorescent cytoplasmic aggregates virtually indistinguishable from that observed before forskolin stimulation.
To test whether the return of fusion protein to the cytoplasmic aggregates reflected a decreased $[cAMP]_i$, cells were treated with a combination of 10 μM forskolin and 100 μM IBMX (n=2) then washed repeatedly (5-8 times) with buffer containing 100 μM IBMX (Fig. 3F). In these experiments, the fusion protein did not return to its prestimulatory localisation after removal of forskolin.

Testing the PKA-F64L-S65T-GFP probe with physiologically relevant agents.

To test the probe's response to receptor activation of adenylate cyclase, BHK cells stably transfected with the glucagon receptor and the PKA-F64L-S65T-GFP probe were exposed to glucagon stimulation. The glucagon receptor is coupled to a G_S protein which activates adenylate cyclase, thereby increasing the cAMP level. In these cells, addition of 100 nM glucagon (n=2) caused the release of the PKA-F64L-S65T-GFP probe from the cytoplasmic aggregates and a resulting translocation of the fusion protein to a more even cytoplasmic distribution within 2-3 min (Fig. 3G). Similar but less pronounced effects were seen at lower glucagon concentrations (n=2, data not shown). Addition of buffer (n=2) had no effect over time (data not shown).
Transiently transfected CHO cells expressing hARα2a and the PKA-F64L-S65T-GFP probe were treated with 10 μM forskolin for 7.5 minutes, then. in the continued presence of forskolin, exposed to 10 μM norepinephrine to stimulate the exogenous adrenoreceptors, which couple to a G_I protein, which inhibit adenylate cyclase. This treatment led to reappearance of fluorescence in the cytoplasmic aggregates indicative of a decrease in $[cAMP]_i$ (Fig. 3H).

Fusion protein translocation correlated with $[cAMP]_i$
As described above, the time it took for a response to come to completion was dependent on the forskolin dose (Fig. 5) In addition the degree of responses was also dose-dependent. To test the PKA-F64L-S65T-GFP fusion protein translocation in a semi high through-put system, CHO cells stably transfected with the PKA-F64L-S65T-GFP fusion was stimulated with buffer and 5 increasing doses of forskolin (n=8). Using the image analysis algorithm described above (Method 1), a dose-response relationship was observed in the range from 0.01-50 μM forskolin (Fig. 6). A half-maximal stimulation was observed at about 2 μM forskolin. In parallel, cells were stimulated with buffer and 8 increasing concentrations of forskolin (n=4) in the range 0.01-50 μM. The amount of cAMP produced was measured in an SPA assay. A steep increase was observed between 1 and 5 μM forskolin coincident with the steepest part of the curve for fusion protein translocation (also Fig. 6).

EXAMPLE 2 Probe for detection of PKC activity

Construction of PKC-GFP fusion:
The probe was constructed by ligating two restriction enzyme treated polymerase chain reaction (PCR) amplification products of the cDNA for murine PKCα (GenBank

Accession number: M25811) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) respectively. Taq® polymerase and the following oligonucleotide primers were used for PCR;

5'mPKCα:
TTggACACAAgCTTTggACACCCTCAggATATggCTgACgTTTACCCggCCAACg
3'mPKCα:
gTCATCTTCTCgAgTCTTTCAggCgCgCCCTACTgCACTTTgCAAgATTgggTgC,
5'F64L-S65T-GFP:
TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTTC,
3'F64L-S65T-GFP:
gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT.

The hybrid DNA strand was inserted into the pZeoSV® mammalian expression vector as a HindIII-XhoI casette as described in example 1.
BHK cells expressing the human M1 receptor under the control of the inducible metallothionine promoter and maintained with the dihydrofolate reductase marker were transfected with the PKCα-F64L-S65T-GFP probe using the calcium phosphate precipitate method in HEPES buffered saline (HBS [pH 7.10]). Stable transfectants were selected using 1000 μg Zeocin®/ml in the growth medium (DMEM with 1000 mg glucose/l, 10 % foetal bovine serum (FBS), 100 μg penicillin-streptomycin mixture ml-1, 2 mM l-glutamine). The hM1 receptor and PKCα-F64L-S65T-GFP fusion protein were maintained with 500 nM methotrexate and 500 μg Zeocin®/ml respectively. 24 hours prior to any experiment, the cells were transferred to HAM F-12 medium with glutamax, 100 μg penicillin-streptomycin mixture ml^{-1} and 0.3 % FBS. This medium relieves selection pressure, gives a low induction of signal transduction pathways and has a low autofluorescence at the relevant wavelength enabling fluorescence microscopy of cells straight from the incubator.

Method 1: Monitoring the PKCα activity in real time:
Digital images of live cells were gathered using a Zeiss Axiovert 135M fluorescence microscope fitted with a 40X, NA: 1.3 oil immersion objective and coupled to a Photometrics CH250 charged coupled device (CCD) camera. The cells were illuminated with a 100 W arc lamp. In the light path was a 470±20 nm excitation filter, a 510 nm dichroic mirror and a 515±15 nm emission filter for minimal image background. The cells were kept and monitored to be at 37°C with a custom built stage heater.

Images were analyzed using the IPLab software package for Macintosh.

Upon stimulation of the M1-BHK cells, stably expressing the PKCα-F64L-S65T-GFP fusion, with carbachol we observed a dose-dependent transient translocation from the cytoplasm to the plasma membrane (Fig. 7a,b,c). Simultaneous measurement of the cytosolic free calcium concentration shows that the carbachol-induced calcium mobilisation precedes the translocation (Fig. 8).

Stepwise procedure for quantification of translocation of PKCα:

The image was corrected for dark current by performing a pixel-by-pixel subtraction of a dark image (an image taken under the same conditions as the actual image, except the camera shutter is not allowed to open).

The image was corrected for non-uniformity of the illumination by performing a pixel-by-pixel ratio with a flat field correction image (an image taken under the same conditions as the actual image of a uniformly fluorescent specimen).

A copy of the image was made in which the edges are identified. The edges in the image are found by a standard edge-detection procedure – convolving the image with a kernel which removes any large-scale unchanging components (i.e.. background) and accentuates any small-scale changes (i.e., sharp edges). This image was then converted to a binary image by threshholding. Objects in the binary image which are too small to represent the edges of cells were discarded. A dilation of the binary image was performed to close any gaps in the image edges. Any edge objects in the image which were in contact with the borders of the image are discarded. This binary image represents the edge mask.

Another copy of image was made via the procedure in step 3. This copy was further processed to detect objects which enclose "holes" and setting all pixels inside the holes to the binary value of the edge, i.e., one. This image represents the whole cell mask.

The original image was masked with the edge mask from step 3 and the sum total of all pixel values is determined.

The original image was masked with the whole cell mask from step 4 and the sum total of all pixel values was determined.

The value from step 5 was divided by the value from step 6 to give the final result, the fraction of fluorescence intensity in the cells which was localized in the edges.

EXAMPLE 3 Probes for detection of mitogen activated protein kinase Erk1 redistribution.

Useful for monitoring signalling pathways involving MAPK, e.g. to identify compounds which modulate the activity of the pathway in living cells.

Erk1, a serine/threonine protein kinase, is a component of a signalling pathway that is activated by e.g. many growth factors.

Probes for detection of ERK-1 activity in real time within living cells:

The extracellular signal regulated kinase (ERK-1, a mitogen activated protein kinase, MAPK) is fused N- or C-terminally to a derivative of GFP. The resulting fusions expressed in different mammalian cells are used for monitoring *in vivo* the nuclear translocation, and thereby the activation, of ERK1 in response to stimuli that activate the MAPK pathway.

The human Erk1 gene (GenBank Accession number: X60188) was amplified using PCR according to standard protocols with primers

Erk1-top

5'-TAGAATTCAACCATGGCGGCGGCGGCGGCG-3'

and Erk1-bottom/+stop

5'-TAGGATCCCTAGGGGGCCTCCAGCACTCC-3'.

The PCR product was digested with restriction enzymes EcoR1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with EcoR1 and BamH1. This produces an EGFP-Erk1 fusion (SEQ ID NOs: 5 and 6) under the control of a CMV promoter.

The plamid containing the EGFP-Erk1 fusion was transfected into HEK293 cells employing the FUGENE transfection reagent (Boehringer Mannheim). Prior to experiments the cells were grown to 80%-90% confluency 8 well chambers in DMEM with 10% FCS. The cells were washed in plain HAM F-12 medium (without FCS), and then incubated for 30-60 minutes in plain HAM F-12 (without FCS) with 100 micromolar PD98059, an inhibitor of MEK1, a kinase which activates Erk1; this step effectively empties the nucleus of EGFP-Erk1. Just before starting the experiment, the HAM F-12 was replaced with Hepes buffer following a wash with Hepes buffer. This removes the PD98059 inhibitor; if blocking of MEK1 is still wanted (e.g. in control experiments), the inhibitor is included in the Hepes buffer.

The experimental setup of the microscope was as described in example 1.

60 images were collected with 10 seconds between each, and with the test compound added after image number 10.

Addition of EGF (1-100 nM) caused within minutes a redistribution of EGFP-Erk1 from the cytoplasm into the nucleus (Fig. 9a,b).

The response was quantitated as described below and a dose-dependent relationship between EGF concentration and nuclear translocation of EGFP-Erk1 was found (Fig. 9c,d). Redistribution of GFP fluorescence is expressed in this example as the change in the ratio value between areas in nuclear versus cytoplasmic compartments of the cell. Each time profile is the average of nuclear to cytoplasmic ratios from six cells in each treatment.

EXAMPLE 4 Probes for detection of Smad2 redistribution.

Useful for monitoring signalling pathways activated by some members of the transforming growth factor-beta family, e.g. to identify compounds which modulate the activity of the pathway in living cells.

Smad 2, a signal transducer, is a component of a signalling pathway that is induced by some members of the TGFbeta family of cytokines.

a) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC-3'

and Smad2-bottom/+stop

5'-GTGGTACCTTATGACATGCTTGAGCAACGCAC-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-C1 (Clontech; Palo Alto; GenBank Accession number U55763) digested with EcoR1 and Acc65I. This produces an EGFP-Smad2 fusion (SEQ ID NOs: 7 and 8) under the control of a CMV promoter.

b) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC-3'

and Smad2-bottom/-stop

5'-GTGGTACCCATGACATGCTTGAGCAACGCAC-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with EcoR1 and Acc65I. This produces a Smad2-EGFP fusion (SEQ ID NOs:9 and 10) under the control of a CMV promoter.

The plasmid containing the EGFP-Smad2 fusion was transfected into HEK293 cells, where it showed a cytoplasmic distribution. Prior to experiments the cells were grown in 8 well Nunc chambers in DMEM with 10% FCS to 80% confluence and starved overnight in HAM F-12 medium without FCS.

For experiments, the HAM F-12 medium was replaced with Hepes buffer pH 7.2. The experimental setup of the microscope was as described in example 1. 90 images were collected with 10 seconds between each, and with the test compound added after image number 5.

After serum starvation of cells, each nucleus contains less GFP fluorescence than the surrounding cytoplasm (Fig. 10a). Addition of TGFbeta caused within minutes a redistribution of EGFP-Smad2 from the cytoplasma into the nucleus (Fig. 10b).

The redistribution of fluorescence within the treated cells was quantified simply as the fractional increase in nuclear fluorescence normalised to the starting value of GFP fluorescence in the nucleus of each unstimulated cell and displayed a dose dependent change in response to TGFβ (fig. 10c).

EXAMPLE 5 Probes for detection of VASP redistribution.

Useful for monitoring signalling pathways involving rearrangement of cytoskeletal elements, e.g. to identify compounds which modulate the activity of the pathway in living cells. VASP, a phosphoprotein, is a component of cytoskeletal structures, which redistributes in response to signals that affect focal adhesions.

The human VASP gene (GenBank Accession number: Z46389) was amplified using PCR according to standard protocols with primers

VASP-top

5'-GGGAAGCTTCCATGAGCGAGACGGTCATC-3'

and VASP-bottom/+stop

5'-CCCGGATCCTCAGGGAGAACCCCGCTTC-3'.

The PCR product was digested with restriction enzymes Hind3 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Hind3and BamH1. This produces an EGFP-VASP fusion (SEQ ID NOs:11 and 12) under the control of a CMV promoter.

The resulting plasmid was transfected into CHO cells expressing the human insulin receptor using the calcium-phosphate transfection method. Prior to experiments, cells were grown in 8 well Nunc chambers and starved overnight in medium without FCS. Experiments are performed in a microscope setup as described in example 1.10% FCS was added to the cells and images were collected. The EGFP-VASP fusion was

redistributed from a somewhat even distribution near the periphery into more localised structures, identified as focal adhesion points (Fig. 11).

EXAMPLE 7 Probes for detection of NFkappaB redistribution.

Useful for monitoring signalling pathways leading to activation of NFkappaB, e.g. to identify compounds which modulate the activity of the pathway in living cells. NFkappaB, an activator of transcription, is a component of signalling pathways that are responsive to a varity of inducers including cytokines, lymphokines, and some immunosuppressive agents.

a) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers
NFkappaB-top
5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC-3'
and NFkappaB-bottom/+stop
5'-GTGGATCCTTAGGAGCTGATCTGACTCAGCAG-3'.
The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Xho1 and BamH1. This produces an EGFP-NFkappaB fusion (SEQ ID NOs:13 and 14) under the control of a CMV promoter.

b) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers
NFkappaB-top
5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC-3'
and NFkappaB-bottom/-stop
5'-GTGGATCCAAGGAGCTGATCTGACTCAGCAG-3'.
The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Xho1 and BamH1. This produces an NFkappaB-EGFP fusion (SEQ ID NOs: 15 and 16) under the control of a CMV promoter.

The resulting plasmids are transfected into a suitable cell line, e.g. Jurkat, in which the EGFP-NFkappaB probe and/or the NFkappaB-EGFP probe should change its cellular distribution from cytoplasmic to nuclear in response to activation of the signalling pathway with e.g. IL-1.

CHO cells stably expressing the insulin receptor and a human NFkB – GFP protein hybrid were stimulated with different concentrations of IL-1 for 1 hour, then washed with a hypoosmotic buffer (TRIS-base 10mM, MgCl2 2mM, PMSF(Phenyl methyl sulfonyl fluoride) 0.5mM, pH 7.4) and placed on the microscope. A sequence of images were acquired during the addition of 0.05% Triton X-100 and subsequent gentle mixing after a short incubation period. The treatment causes the cell membranes to rupture leaving the fraction of NFkB-GFP that has translocated to the nucleus behind whereas the cytoplasmic amount of the probe leaves the cells more quickly and immediately becomes infinitely diluted in the surrounding medium (out of focus - this part of the total fluorescence from the probe is thereby lost). At a defined time point before and after this treatment a total intensity value for the whole image was extracted. To normalize each experiment, the after value was divided by the before value, meaning that a higher ratio was found in cells where more NFkB had translocated to the nucleus and thereby contributed to the total fluorescence after permeabilisation. the actual data from such an experiment run in duplicate is shown in Figure 18.

Conclusion: the present protocol is a good example of the possibility of revealing translocation of a fluorescent probe from the cytosol to the nucleus or translocation from the nucleus to the cytosol by using a measurement immediately before and after plasma membrane permeabilisation recorded a s an image sequence.

EXAMPLE 8 real-time redistribution of protein kinase C α

Measurement of the real-time redistribution of protein kinase C α isoform-GFP fusion (PKCα-GFP, SEQ ID NOs: 3 and 4) in response to carbamylcholine stimulation of the muscarinic M1 receptor; 96 parallel redistribution measurements in microtiter plates.

BHK cells were stably expressing a recombinant human muscarinic type 1 receptor, under the selection with 500 μg/ml Methotrexate, and also a PKCα-GFP construct (KaA 048), under the selection of 500 nM Zeocin. The cells were grown in 96-well plates (Packard ViewPlate, black with transparent bottom), washed and preincubated in a Hank's Buffered Salt solution (HBSS) without phenol red, with 20 mM HEPES and 5.5 mM glucose.

The plate was measured in a FLIPR™ (Fluorescence Imaging Plate Reader) from Molecular Devices. The 488 nm emission line from an argon ion laser, run at between 0.4 and 0.8 W output, was used to excite fluorescence form the GFP. Emission wavelengths were collected through a 510 to 565 nm band pass filter.

The cells were challenged with three doses of carbamylcholine, an M1 receptor agonist known from previous studies to give a microscopically detectable redistribution of the PKCα-GFP construct [(Almholt *et al.* 1997)]. Measurements were made every 10 seconds for 5 minutes. After data handling including normalisation of baseline fluorescence for the different wells. background subtraction and averaging the 6 wells used for each concentration the data presented in figure 14 were obtained. It can clearly be seen (Fig 12) that carbamylcholine gave a time- and dose-dependent, and transient, decrease in fluorescence very similar to the time- and dose-dependent profile seen in microscopic fluorescence measurements [(see Almholt *et al.* 1997)]. This experiment was repeated twice on the same batch of cells with similar results.

EXAMPLE 9 real-time redistribution of cyclic-AMP dependent protein kinase catalytic subuit-GFP fusion

Measurement of the real-time redistribution of cyclic-AMP dependent protein kinase catalytic subuit-GFP fusion (C-GFP^{LT} SEQ ID NOs: 1 and 2) in response to forskolin stimulation of the adenylate cyclase: 96 parallel redistribution measurements in microtiter plates.

CHO cells were stably transfected with hybrid DNA for the PKA catalytic subunit-F64L+S65T GFP (C-GFP^{LT}) fusion protein, and were typically under continuous selection with 1000 μg/ml zeocin (Invitrogen). The cells were grown without selection for 2 days in 96-well plates (Packard ViewPlate. black with transparent bottom), washed and preincubated in a Hank's Buffered Salt solution (HBSS) without phenol red, with 20 mM HEPES and 5.5 mM glucose.

The plate was measured in a FLIPR™ (Fluorescence Imaging Plate Reader) from Molecular Devices. The 488 nm emission line from an argon ion laser, run at between 0.4 and 0.8 W output, was used to excite fluorescence from the GFP. Emission wavelengths were collected through a 510 to 565 nm band pass filter.

The cells were challenged with three doses of forskolin (Fig 13), an adenylate cyclase agonist known from previous studies to give a microscopically detectable redistribution of the C-GFP^{LT} construct. Measurements were made every 10 seconds for over 6 minutes from the point of addition of forskolin. After data handling including normalisation of baseline fluorescence for the different wells, background subtraction and averaging the 6 wells used for each concentration the data presented below were obtained. It can clearly be seen in figure 15 that forskolin gave a time- and dose-dependent decrease in fluorescence very similar to the time- and dose-dependent profile seen in microscopic

fluorescence measurements. This experiment was repeated twice on the same batch of cells with similar results. As can be seen in figure 14, a more extensive dose-response test gives at hand that this method is both sensitive and reproducible enough to use as the basis for a high throughput screening assay.

EXAMPLE 10 cyclic-AMP dependent protein kinase catalytic subuit-GFP fusion

Measurement of the redistribution response of cyclic-AMP dependent protein kinase catalytic subuit-GFP fusion (C-GFP^{LT} SEQ ID NOs: 1 and 2) after forskolin stimulation of the adenylate cyclase; measurement of the change in total fluorescence upon permeabilisation of agonist-treated cells.

CHO cells were stably transfected with hybrid DNA for the PKA catalytic subunit-F64L+S65T GFP (C-GFP^{LT}) fusion protein, and were typically under continuous selection with 1000 μg/ml zeocin (Invitrogen). For the experiments reported here, cells were grown without selection to 90% confluence in 8-well tissue culture-treated Lab-Tek® chambered coverglass units (chambers, obtained from Nunc, Inc. Illinois, USA). Immediately prior to the experiment growth medium was washed from the cells and replaced with 200 μl HEPES buffer per well.

For the results reported here, chambers were measured using a cooled CCD camera (KAF1400 chip, Photometrics Ltd., USA) attached to an inverted microscope (Diaphot 300, Nikon, Japan) equipped with a x40 oil-immersion Fluar lens, NA 1.4. Cells were illuminated with 450-490 nm light from a 50 W HBO lamp, and emitted light collected between 510-560 nm.

The cells were challenged with four doses of forskolin, an adenylate cyclase agonist known from previous studies to give a microscopically detectable redistribution of the C-GFP^{LT} construct. Images were collected at 10-second intervals for a period of 10 minutes for each treatment. Six minutes after the addition of forskolin or buffer control, Triton-X100 was added to a final concentration of 0.1%. The detergent releases freely mobile C-GFP^{LT} from the cells. The change in fluorescence resulting from this loss was measured after 1 minute of equilibration. After data handling including background subtraction and normalisation to pre-detergent values, the data presented in figure 16 were obtained. Permeabilisation caused decreases in fluorescence, the magnitude of which were dependent on the forskolin treatments. This experiment was repeated twice on the same batch of cells with similar results.

EXAMPLE 11 Probes for detection of PKCβ1 redistribution.

Useful for monitoring signalling pathways involving Protein Kinase C, e.g. to identify compounds which modulate the activity of the pathway in living cells.

PKCbeta1, a serine/threonine protein kinase, is closely related to PKCalpha and PKCbeta2 but not identical; it is a component of a signalling pathway which is activated by elevation of intracellular calcium concomitant with an increase in diacylglycerol species.

a) The human PKCbeta1 gene (GenBank Accession number: X06318) was amplified using PCR according to standard protocols with primers

PKCβ1-top

GTCTCGAGGCAAGATGGCTGACCC

and PKCβ1-bottom

GTGGATCCCTACACATTAATGACAAACTCTGGG.

The PCR product was digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Xho1 and BamH1. This produces an EGFP-PKCβ1 fusion (SEQ ID NOs: 17 and 18) under the control of a CMV promoter.

b) CHO cells stably expressing the human insulin receptor and human PKC beta 1 labeled with EGFP were investigated in the microscope. A dose-response was created where a set of cells were imaged over time for each concentration. The changes in fluorescence were calculated as AUC for 4 min of stimulation.

It can be seen in figure 16 that using microscopic measurements, redistribution of human PKC beta 1 – EGFP can only be detected if a subregion of each cell is analysed. The event is clearly visible when the image series is viewed as a movie but if the whole image changes in fluorescence or the changes in fluorescence in entire cells are analysed the redistribution cannot be detected.

CHO cells stably expressing the human insulin receptor and human PKC beta 1 labelled with EGFP were investigated in the FLIPR™. A dose-response was created where six separate wells were imaged over time for each concentration. The changes in fluorescence were calculated as AUC for 5 min of stimulation. As shown in figure 17 redistribution of human PKC beta 1 – EGFP can be detected in the FLIPR™ instrument despite the fact that the whole wells, containing around 50-100 000 cells, are illuminated and imaged with a resolution far below what is needed to resolve single cells or subcellular compartments. This phenomenon can clearly not be predicted from the

microscope data in Figure 16. Based on these observations it is clear that a screening assay can be established in the FLIPR™ instrument. It might even be possible to establish a high throughput screening assay with further optimisation.

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International Patent Application No. PCT/DK99/00562
Our ref: 22129PC1, Improved method for redistribution
BioImage A/S

CLAIMS

1. A method for extracting quantitative information relating to an influence on redistribution of at least one component in the cell in mechanically intact or permeabilised living cells, the method comprising

recording variation in spatially distributed light emitted from a luminophore, the luminophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence,

as a change in light intensity wherein the illumination is provided by a laser which is scanned in a raster fashion over some or all of the spatial limitations being measured, the scanning taking place at a rate substantially faster than the measurement process such that the illumination appears to the measurement process to be continuous in time and spatially uniform over the region being measured.

2. A method according to claim 1, wherein the quantitative information which is indicative of the degree of the cellular response to the influence or the result of the influence on the sub-cellular component is extracted from the recorded variation according to a predetermined calibration based on responses or results, recorded in the same manner, to known degrees of a relevant specific influence.

3. A method according to claims 1 or 2, wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. A method according to any of claims 1-3, wherein the cells comprise a group of cells contained within a spatial limitation.

5. A method according to any of claims 1-4, wherein the cells comprise multiple groups of cells contained within multiple spatial limitations.

6. A method according to any of claims 1-5, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

7. A method according to claim 6, wherein the spatial limitations are wells in a plate of microtiter type.

8. A method according to any of claims 1-7, wherein the redistribution results in quenching of fluorescence, the quenching being measured as a decrease in the intensity of the fluorescence.

9. A method according to any of claims 1-8, wherein the redistribution results in energy transfer, the energy transfer being measured as a change in the intensity of the luminescence.

10. A method according to any of claims 1-8, wherein the intensity of the light being recorded is a function of the fluorescence lifetime, polarisation, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

11. A method according to any of claims 1-10, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. A method according to any of claims 1-11, wherein the flourescence comes from a fluorophore encoded by and expressed from a nucleotide sequence harboured in the cells.

13. A method according to any of the preceding claims, wherein the flourescence comes from a luminescent polypeptide, such as GFP.

14. A method according to any of the preceding claims, wherein the luminescent polypeptide could be a GFP selected from the group consisting of green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.

15. A method according to any of claims 1-14, wherein the cells are selected from the group consisting of fungal cells, such as yeast cells; invertebrate cells including insect cells; and vertebrate cells, such as mammalian cells.

16. A method according to claim 15, wherein the mechanically intact or permeabilised living cells are mammalian cells which, during the time period over which the influence is observed, are incubated at a temperature of 30°C or above, preferably at a temperature of

from 32°C to 39°C, more preferably at a temperature of from 35°C to 38°C, and most preferably at a temperature of about 37°C.

17. A method according to any of claims 1-16, used as a screening program.

18. A method according claim 17, wherein the method is a screening program for the identification of a biologically active substance that directly or indirectly affects an intracellular signalling pathway and is potentially useful as a medicament, wherein the result of the individual measurement of each substance being screened which indicates its potential biological activity is based on measurement of the redistribution of spatially resolved luminescence in living cells and which undergoes a change in distribution upon activation of an intracellular signalling pathway.

19. A method according to claim 17, wherein the method is a screening program for the identification of a biologically toxic substance as defined herein that exerts its toxic effect by interfering with an intracellular signalling pathway, wherein the result of the individual measurement of each substance being screened which indicates its potential biologically toxic activity is based on measurement of the redistribution of said fluorescent probe in living cells and which undergoes a change in distribution upon activation of an intracellular signalling pathway.

20. A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to any of claims 1-19.

Figure 1

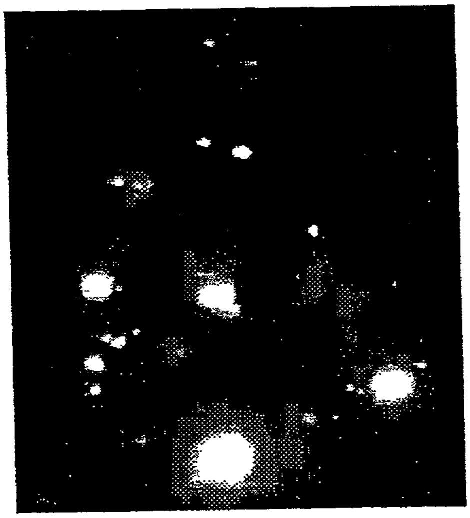


Fig. 1 a

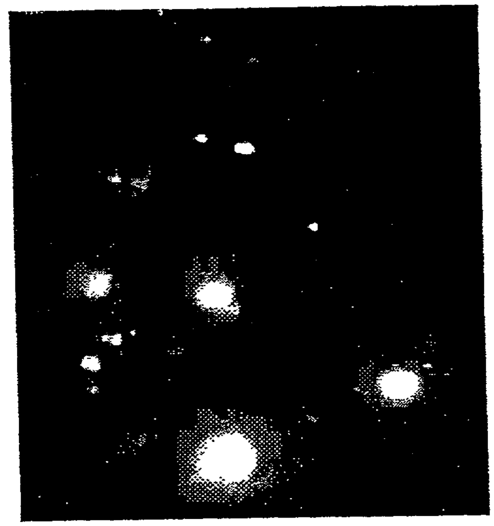


Fig. 1 b

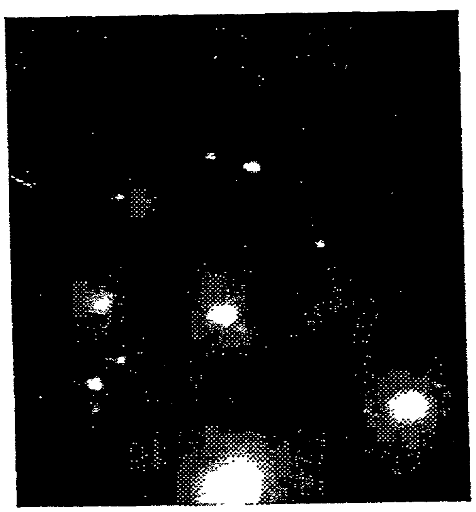


Fig. 1 c

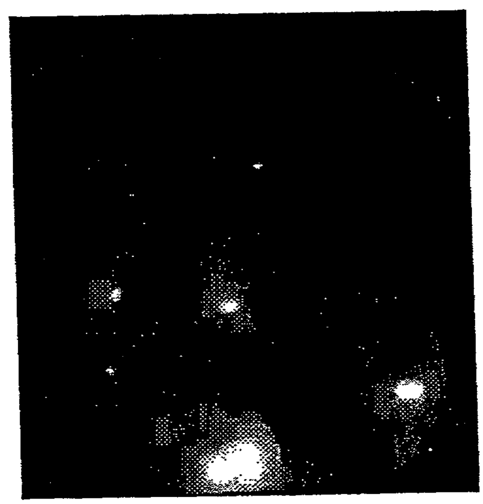


Fig. 1 d

Fig. 1

09/807345

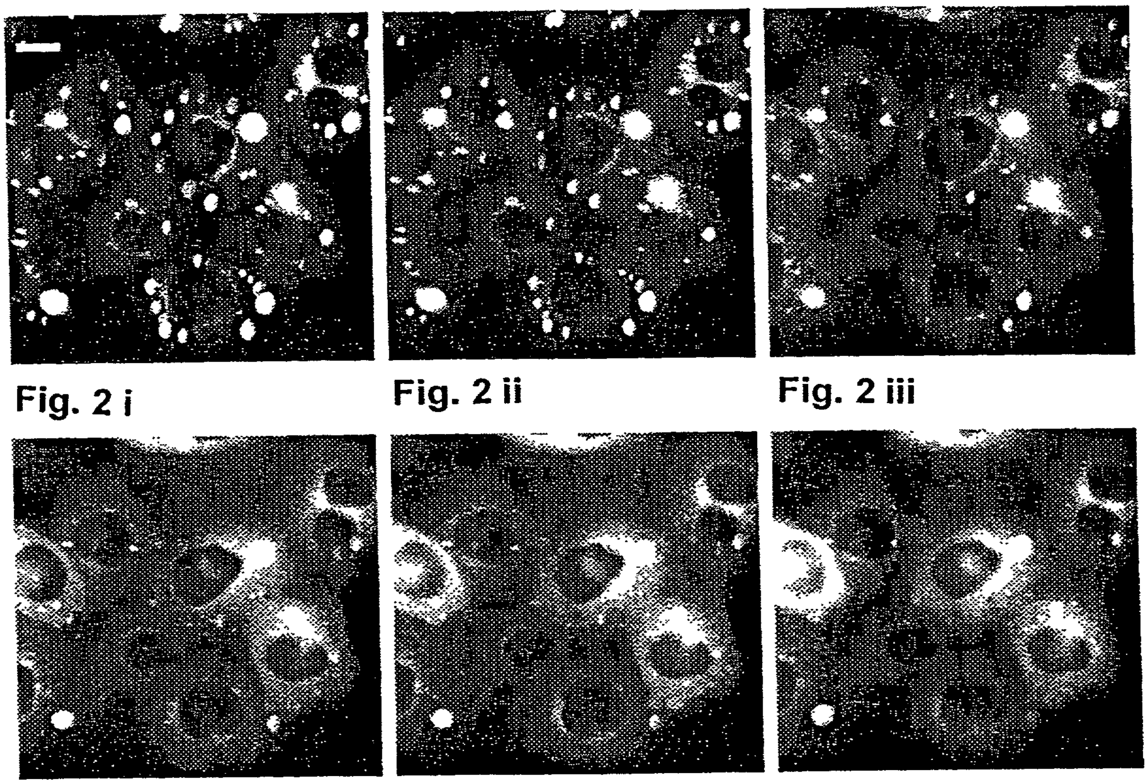


Fig. 2 i Fig. 2 ii Fig. 2 iii

Fig. 2 iv Fig. 2 v Fig. 2 vi

Fig. 2

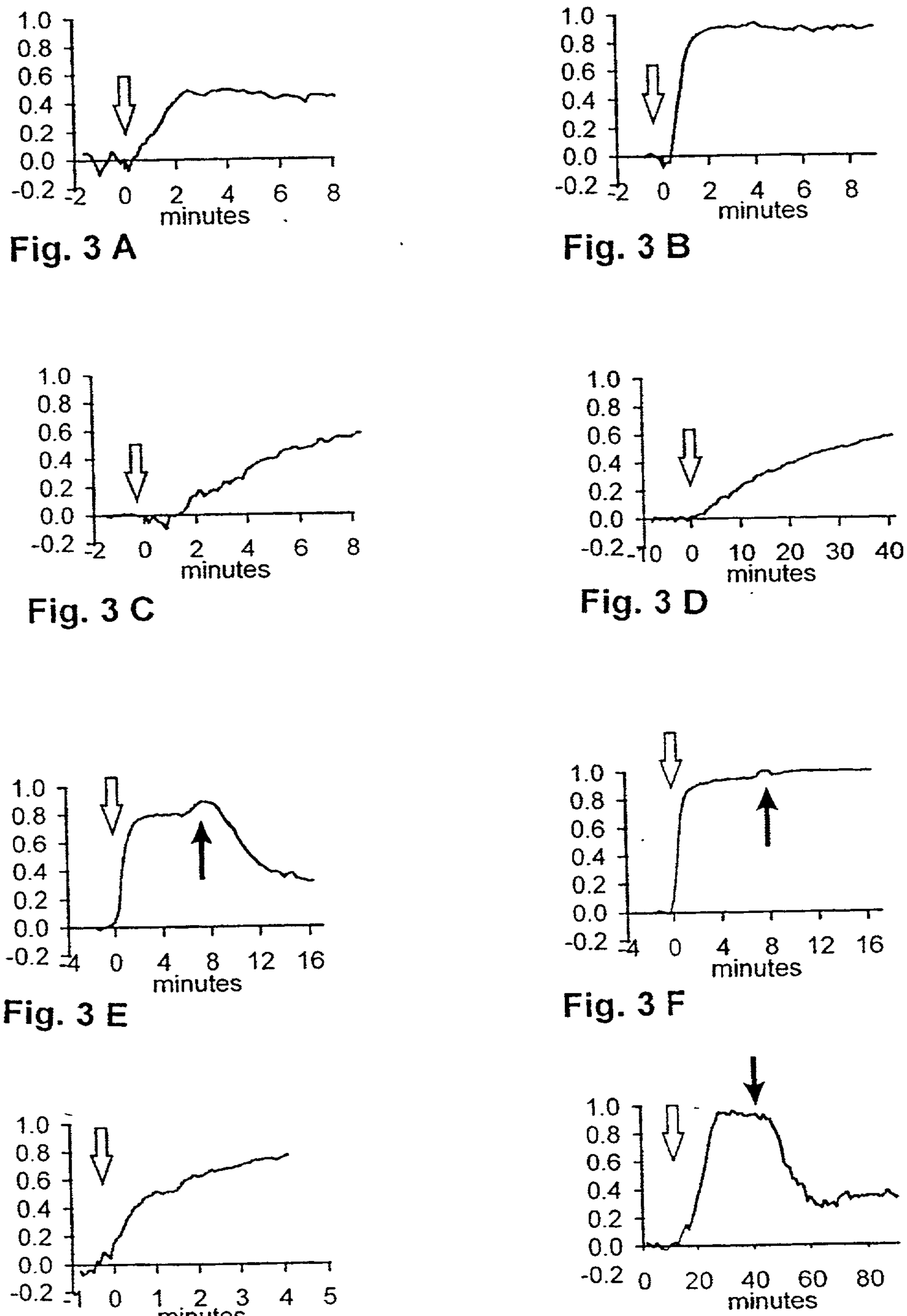


Fig. 3 A

Fig. 3 B

Fig. 3 C

Fig. 3 D

Fig. 3 E

Fig. 3 F

Fig. 3 G

Fig. 3 H

Fig. 3

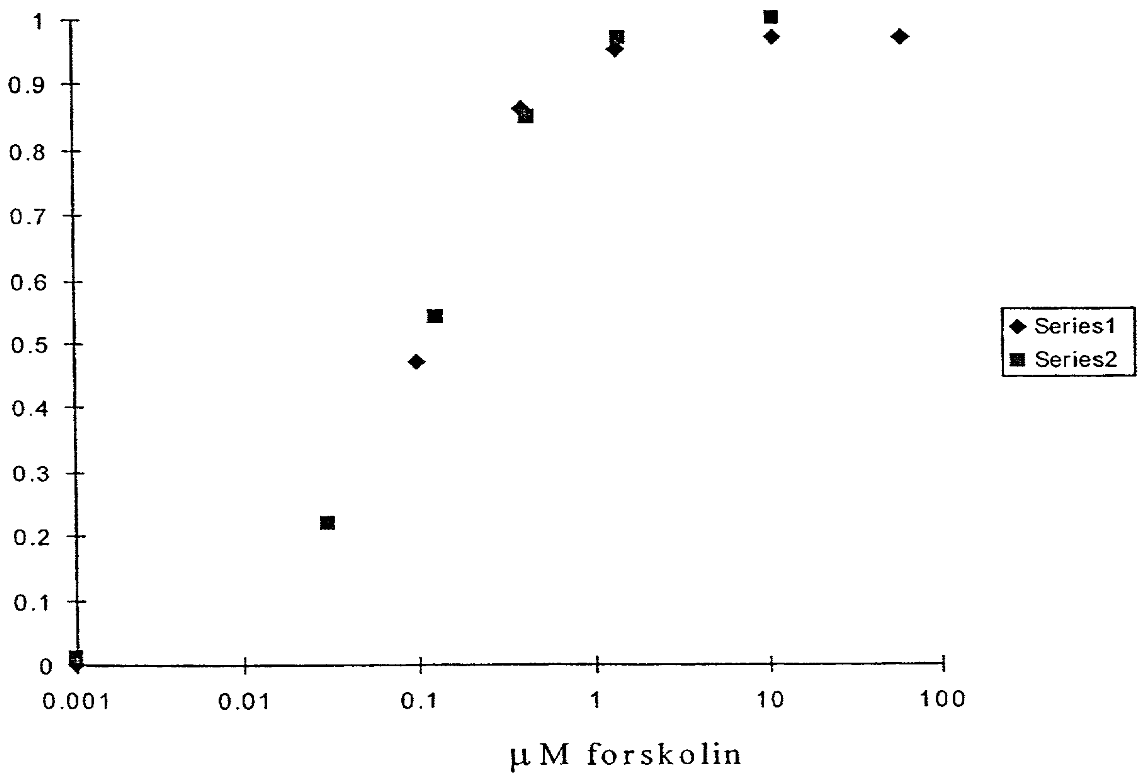
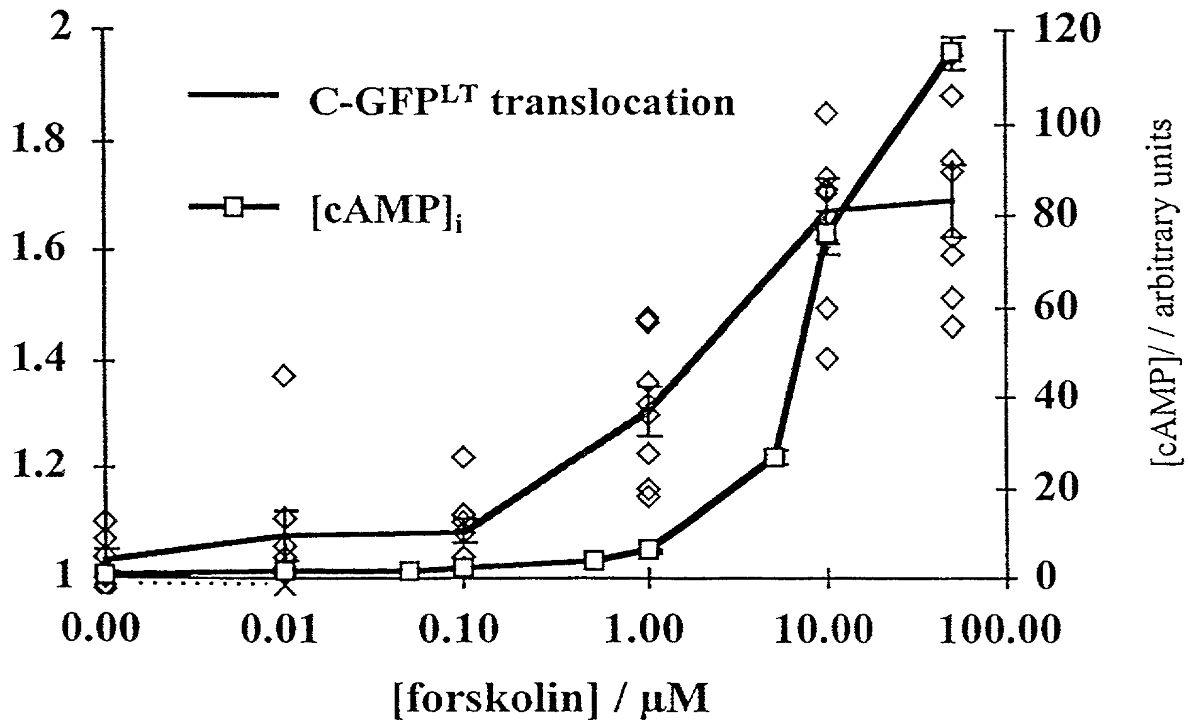

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
µM forskolin
Series1
Series2

Fig. 4

[forskolin]μM	$t_{1/2max}$/ s	t_{max}/ s
1	115±21	310±31
10	69±14	224±47
50	47±10	125±28

Fig. 5



2
1.8
1.6
1.4
1.2
1
C-GFP^LT translocation
[cAMP]i
120
100
80
60
40
20
0
[cAMP]/ / arbitrary units
0.00
0.01
0.10
1.00
10.00
100.00
[forskolin] / µM

Fig. 6

Fig. 7 a

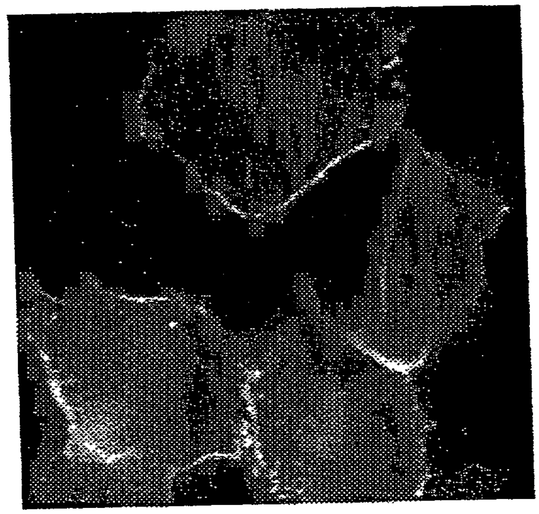
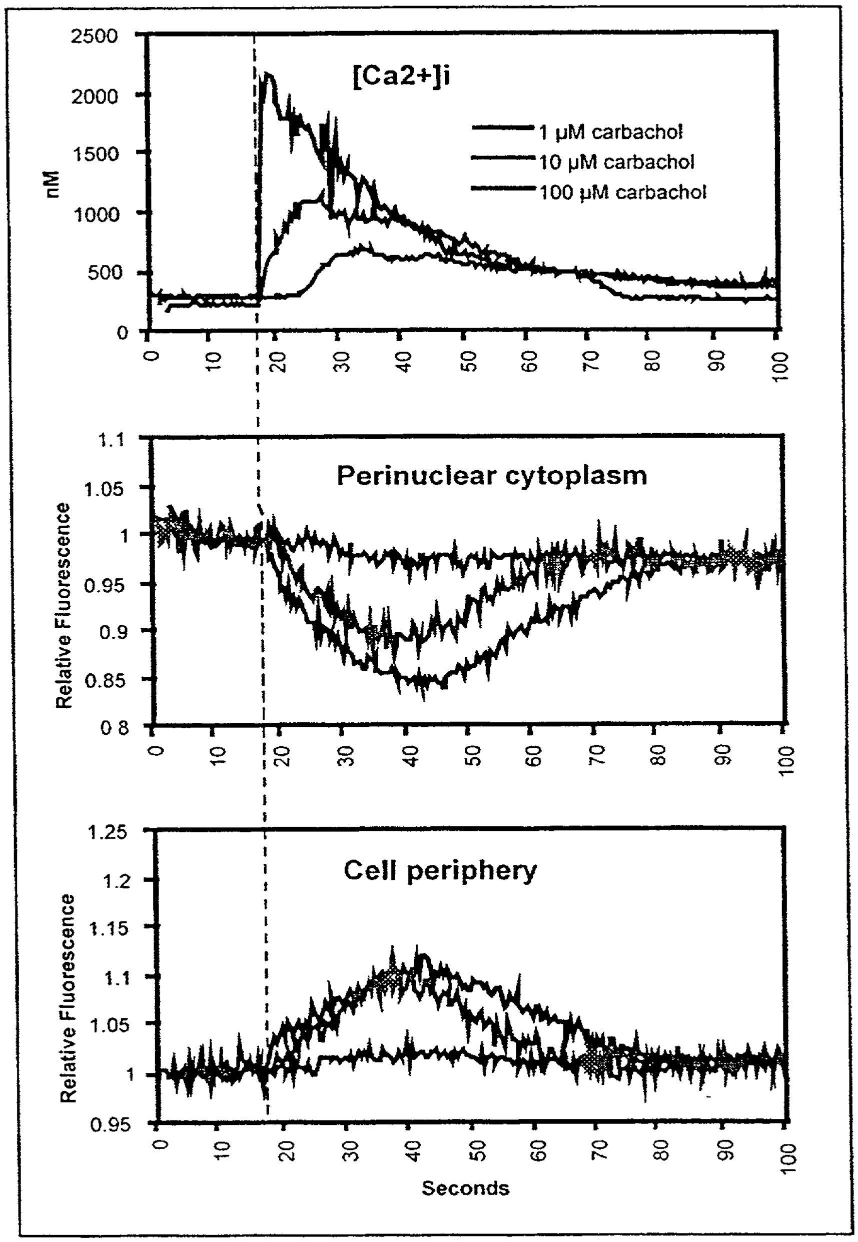


Fig. 7 b

Fig. 7 c

Fig. 7


[Ca2+]i
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10 μM carbachol
100 μM carbachol
nM
Perinuclear cytoplasm
Relative Fluorescence
Cell periphery
Relative Fluorescence
Seconds

Fig. 8

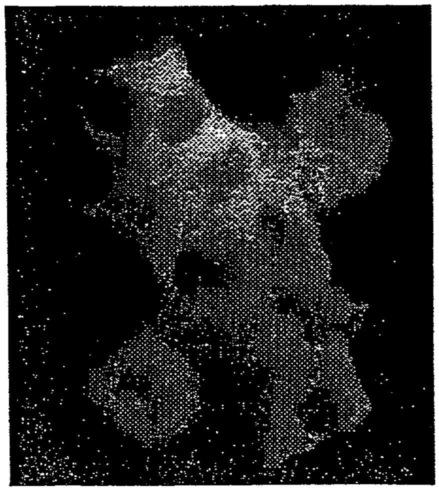

Fig. 9 a

Fig. 9 b

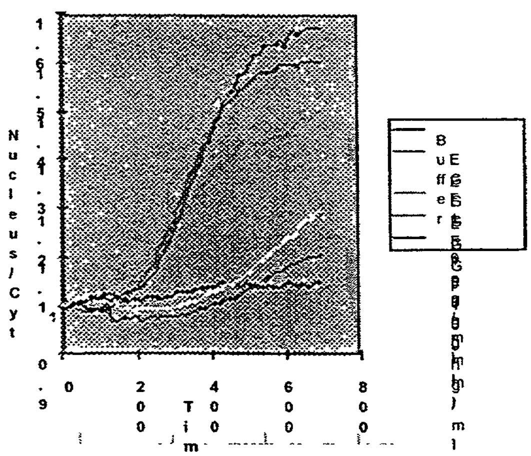

Fig. 9 c

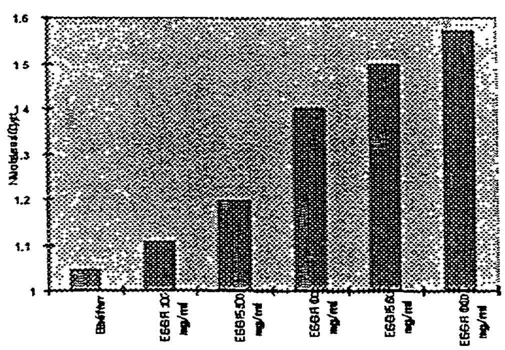

Fig. 9 d

Fig. 9

Fig. 10 a

Fig. 10 b

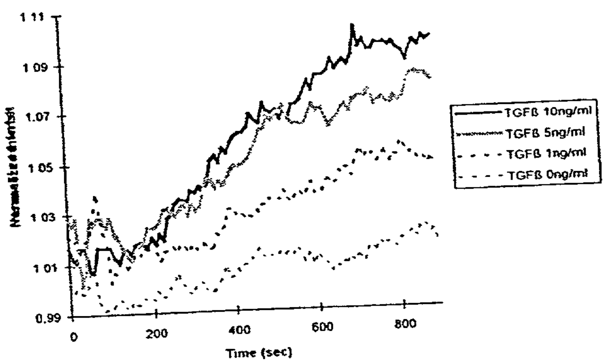

Fig. 10 c

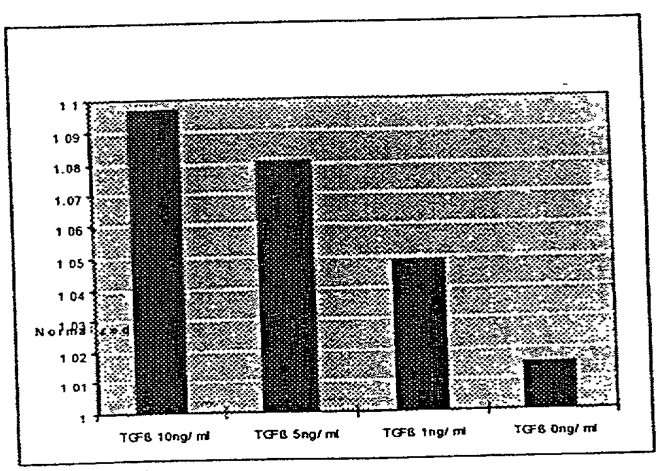

Fig. 10

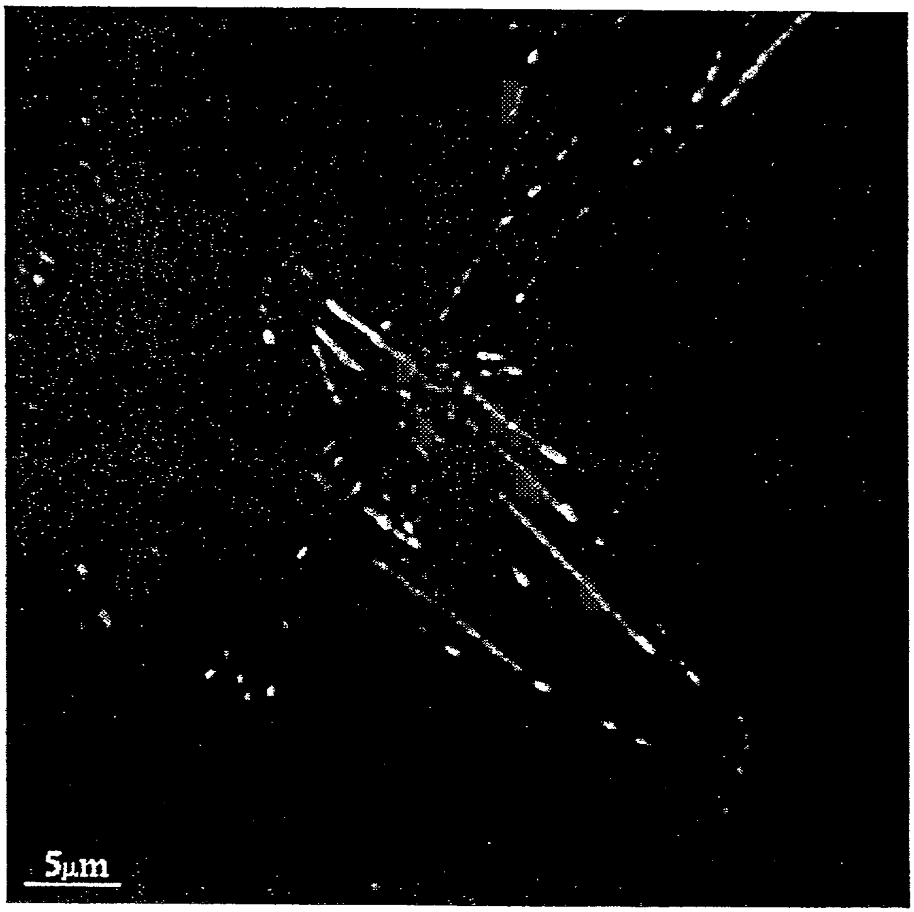

Fig. 11

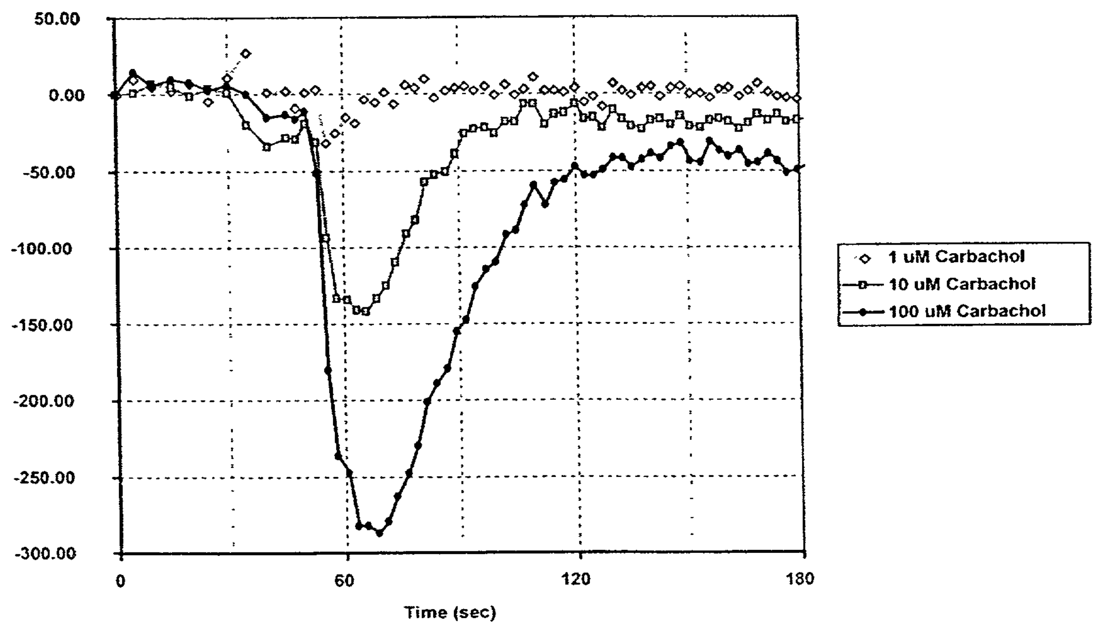
50.00
0.00
-50.00
-100.00
-150.00
-200.00
-250.00
-300.00
0
60
120
180
Time (sec)
1 uM Carbachol
10 uM Carbachol
100 uM Carbachol

Fig. 12

09/807345

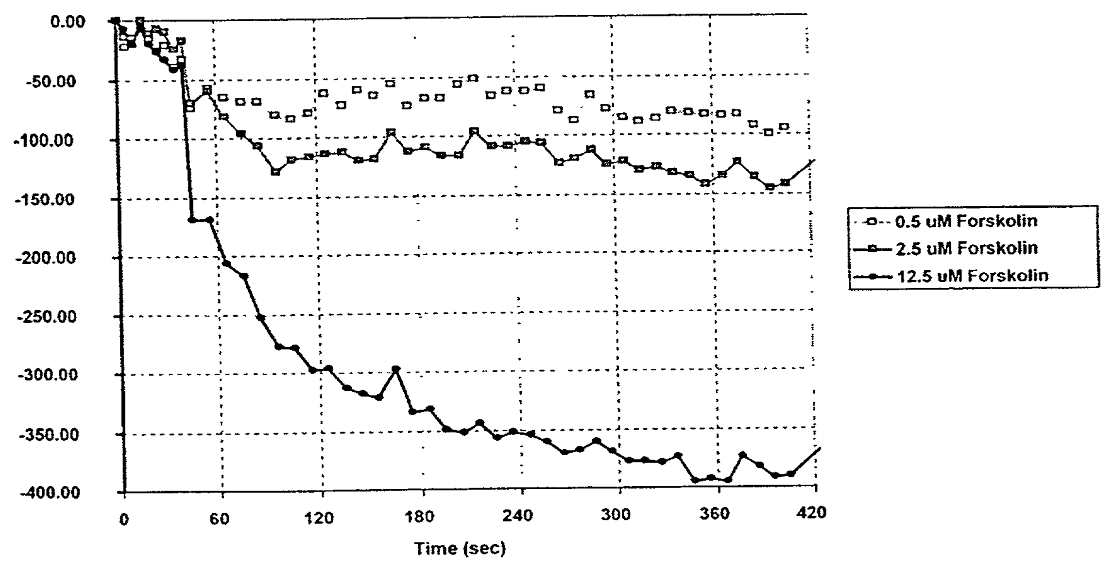

Fig. 13

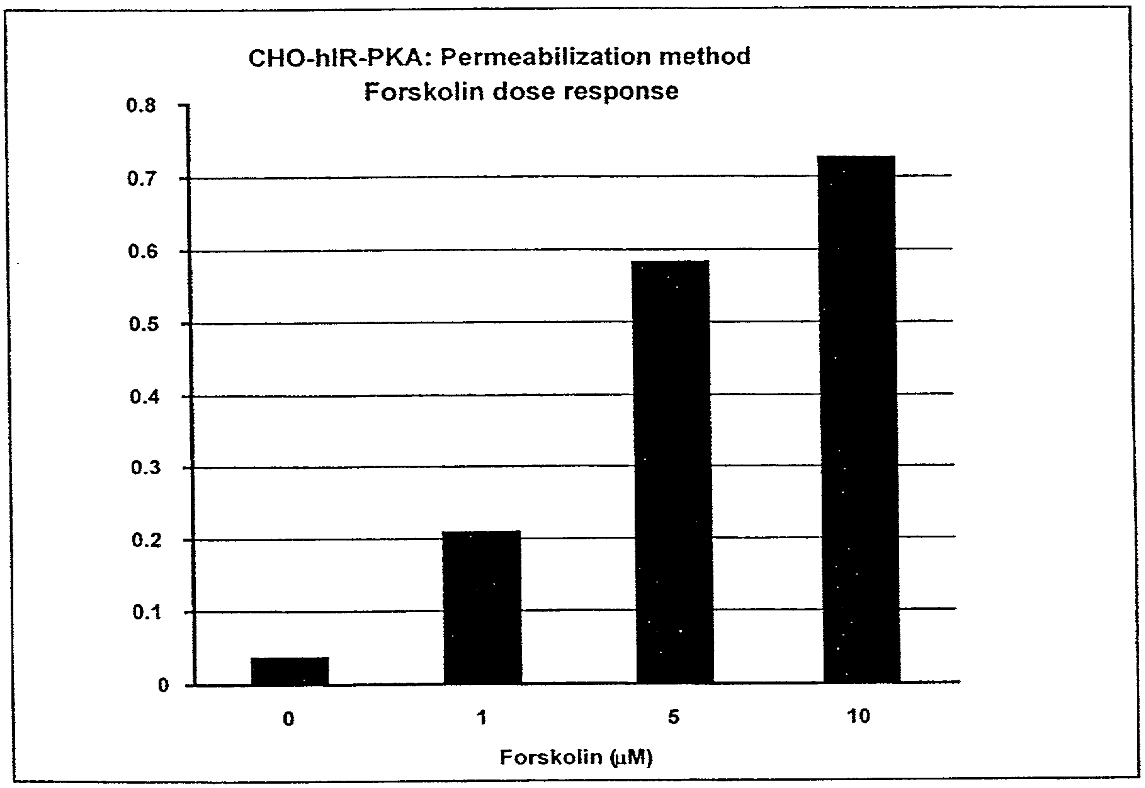
CHO-hIR-PKA: Permeabilization method
Forskolin dose response
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
5
10
Forskolin (µM)

Fig. 14

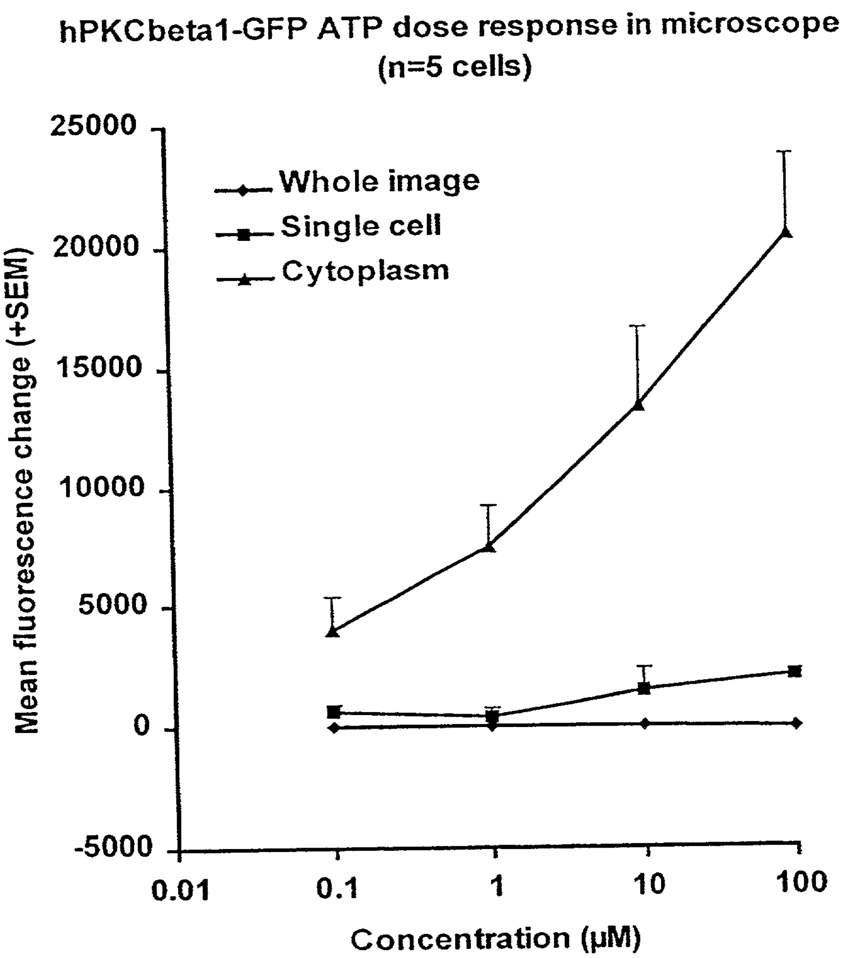
hPKCbeta1-GFP ATP dose response in microscope
(n=5 cells)
Whole image
Single cell
Cytoplasm
Mean fluorescence change (+SEM)
25000
20000
15000
10000
5000
0
-5000
0.01
0.1
1
10
100
Concentration (μM)

Fig. 15

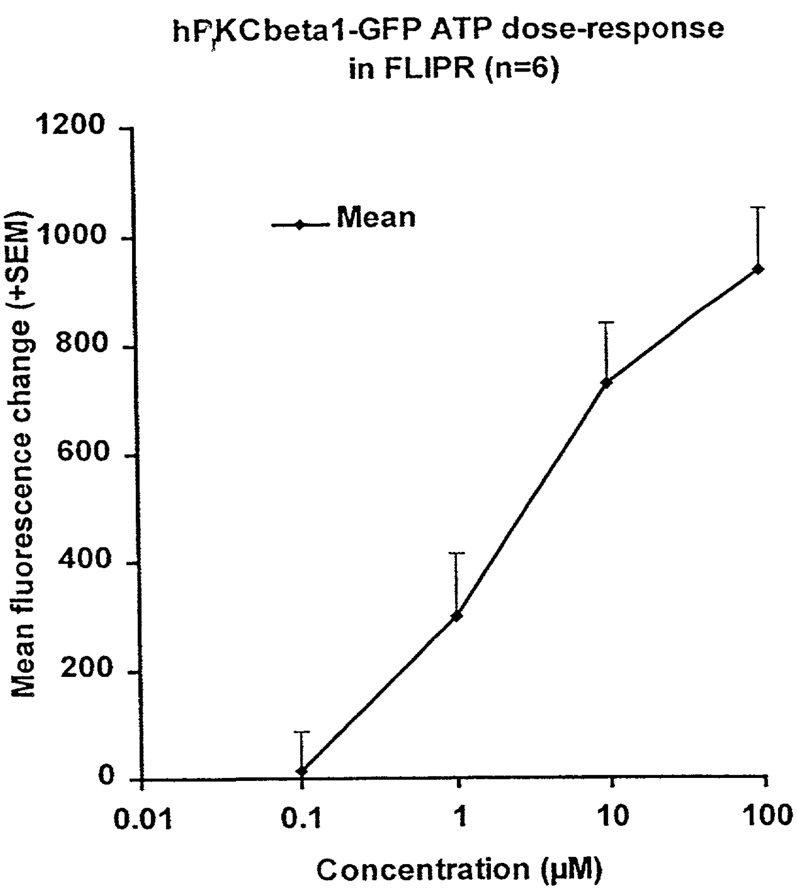
hPKCbeta1-GFP ATP dose-response
in FLIPR (n=6)
Mean
Mean fluorescence change (+SEM)
1200
1000
800
600
400
200
0
0.01
0.1
1
10
100
Concentration (μM)

Fig. 16

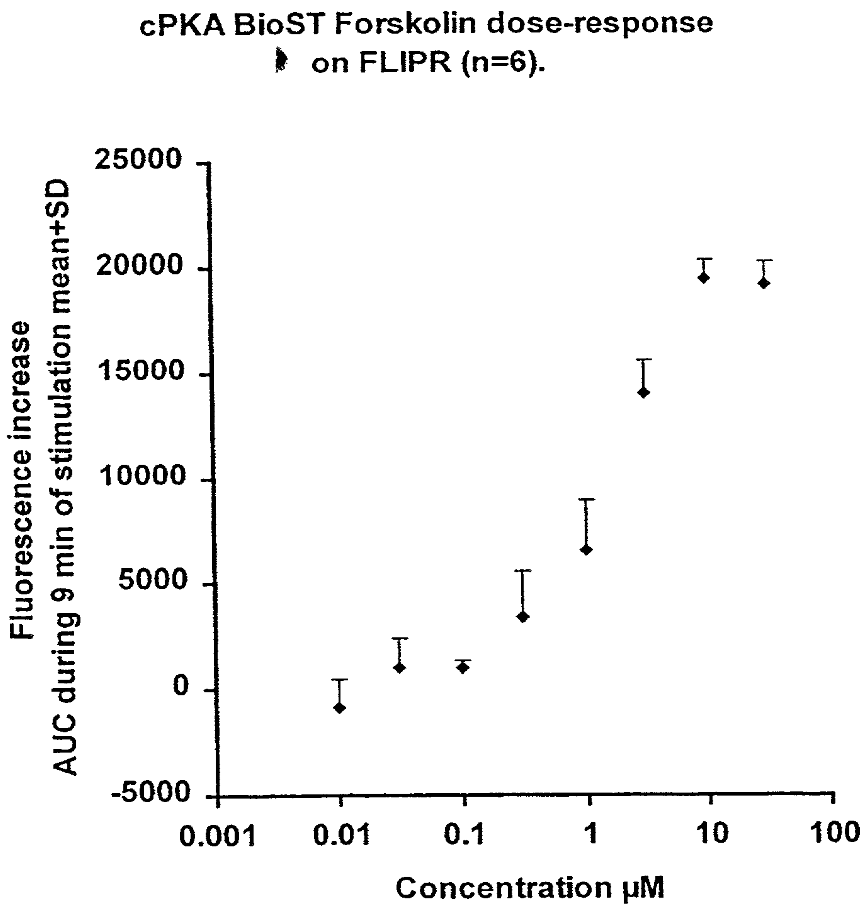
cPKA BioST Forskolin dose-response
on FLIPR (n=6).
Fluorescence increase
AUC during 9 min of stimulation mean+SD
25000
20000
15000
10000
5000
0
-5000
0.001
0.01
0.1
1
10
100
Concentration µM

Fig. 17

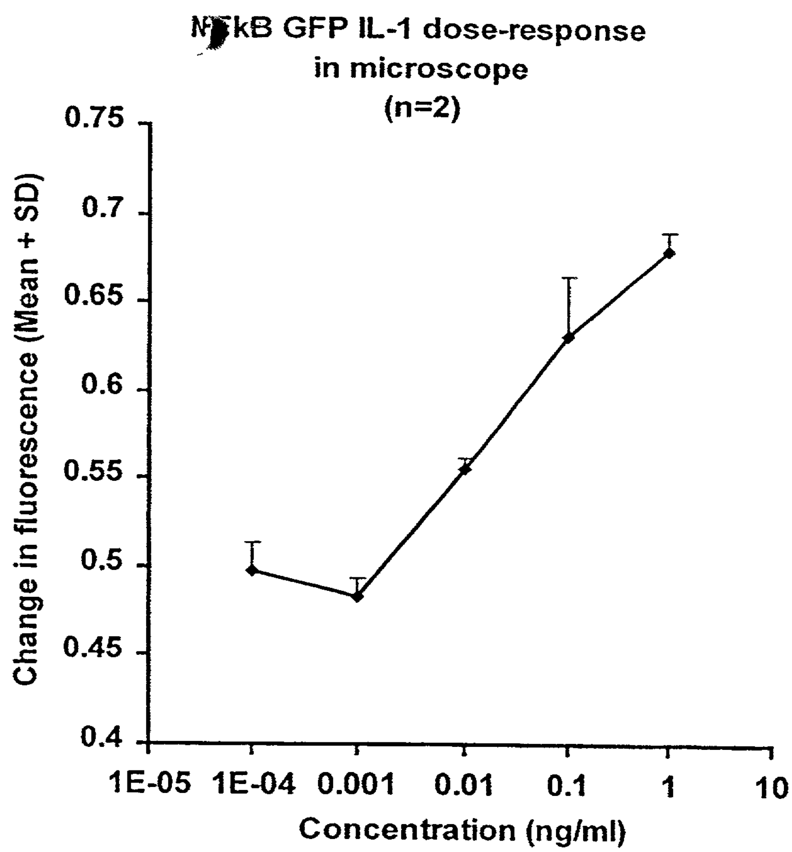
NFkB GFP IL-1 dose-response
in microscope
(n=2)
Change in fluorescence (Mean + SD)
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
1E-05
1E-04
0.001
0.01
0.1
1
10
Concentration (ng/ml)

Fig. 18

Attorney Docket No. 0459-0571P

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PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
the specification was filed on ______ as
United States Application Number ______;
and amended on ______ (if applicable) and/or
the specification was filed on October 15, 1999 as PCT
International Application Number PCT/DK99/00562; and was
amended under PCT Article 34 on November 11, 2000 (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
PA 1998 01320 (Number)	Denmark (Country)	October 15, 1998 (Month/Day/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional Application(s): (if any)

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Insert Requested Information: (if appropriate)

Country	Application Number	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S. Application(s): (if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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Leonard R. Svensson	(Reg. No. 30,330)	Terry L. Clark	(Reg. No. 32,644)
Andrew D. Meikle	(Reg. No. 32,868)	Marc S. Weiner	(Reg. No. 32,181)
Joe McKinney Muncy	(Reg. No. 32,334)	Donald J. Daley	(Reg. No. 34,313)
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full Name of First or Sole Inventor: Insert Name of Inventor → Insert Date This Document is Signed → Insert Residence → Insert Citizenship → Insert Post Office Address →

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Full Name of Ninth Inventor, if any: see above

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Residence (City, State & Country)		CITIZENSHIP
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Full Name of Twelfth Inventor, if any: see above

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Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

*DATE OF SIGNATURE

SEQUENCE LISTING

<110> ARKHAMMAR, Per O. G. et al.

<120> AN IMPROVED METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE

<130> 0459-0571P

<140> 09/807,345
<141> 2001-04-12

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Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser
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Thr Leu Gly Thr Gly Ser Phe Gly Arg Val Met Leu Val Lys His Lys
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Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
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Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu
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Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys
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Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly
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Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
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Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
545                 550                 555                 560

Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu
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Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
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gcc aac cgc ttc gcc cgc aaa ggg gcg ctg agg cag aag aac gtg cat        96
Ala Asn Arg Phe Ala Arg Lys Gly Ala Leu Arg Gln Lys Asn Val His
            20                  25                  30

gag gtg aaa gac cac aaa ttc atc gcc cgc ttc ttc aag caa ccc acc       144
Glu Val Lys Asp His Lys Phe Ile Ala Arg Phe Phe Lys Gln Pro Thr
        35                  40                  45

ttc tgc agc cac tgc acc gac ttc atc tgg ggg ttt ggg aaa caa ggc       192
Phe Cys Ser His Cys Thr Asp Phe Ile Trp Gly Phe Gly Lys Gln Gly
    50                  55                  60

ttc cag tgc caa gtt tgc tgt ttt gtg gtt cat aag agg tgc cat gag       240
Phe Gln Cys Gln Val Cys Cys Phe Val Val His Lys Arg Cys His Glu
65                  70                  75                  80

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Phe Val Thr Phe Ser Cys Pro Gly Ala Asp Lys Gly Pro Asp Thr Asp
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gac ccc agg agc aag cac aag ttc aaa atc cac aca tac gga agc cct       336
Asp Pro Arg Ser Lys His Lys Phe Lys Ile His Thr Tyr Gly Ser Pro
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acc ttc tgt gat cac tgt ggg tcc ctg ctc tat gga ctt atc cac caa       384
Thr Phe Cys Asp His Cys Gly Ser Leu Leu Tyr Gly Leu Ile His Gln
        115                 120                 125

ggg atg aaa tgt gac acc tgc gac atg aat gtt cac aac cag tgt gtg       432
Gly Met Lys Cys Asp Thr Cys Asp Met Asn Val His Asn Gln Cys Val
    130                 135                 140

atc aat gac cct agc ctc tgc gga atg gat cac aca gag aag agg ggg       480
Ile Asn Asp Pro Ser Leu Cys Gly Met Asp His Thr Glu Lys Arg Gly
145                 150                 155                 160
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cgg att tat ctg aag gct gag gtc act gat gaa aag ctc cac gtc acg 528
Arg Ile Tyr Leu Lys Ala Glu Val Thr Asp Glu Lys Leu His Val Thr
165 170 175

gta cga gat gca aaa aat cta atc cct atg gat cca aat ggg ctt tcg 576
Val Arg Asp Ala Lys Asn Leu Ile Pro Met Asp Pro Asn Gly Leu Ser
180 185 190

gat cct tat gtg aag ctg aaa cta atc cct gac ccc aag aat gag agc 624
Asp Pro Tyr Val Lys Leu Lys Leu Ile Pro Asp Pro Lys Asn Glu Ser
195 200 205

aaa cag aaa acc aaa acc atc cgc tcc aac ctg aat cct cag tgg aat 672
Lys Gln Lys Thr Lys Thr Ile Arg Ser Asn Leu Asn Pro Gln Trp Asn
210 215 220

gag tcc ttc acg ttc aaa tta aaa cct tca gac aaa gac cgg cga ctg 720
Glu Ser Phe Thr Phe Lys Leu Lys Pro Ser Asp Lys Asp Arg Arg Leu
225 230 235 240

tct gta gaa atc tgg gac tgg gat cgg acg act cgg aat gac ttc atg 768
Ser Val Glu Ile Trp Asp Trp Asp Arg Thr Thr Arg Asn Asp Phe Met
245 250 255

gga tcc ctt tcc ttt ggt gtc tca gag cta atg aag atg ccg gcc agt 816
Gly Ser Leu Ser Phe Gly Val Ser Glu Leu Met Lys Met Pro Ala Ser
260 265 270

gga tgg tat aaa gct cac aac caa gaa gag ggc gaa tat tac aac gtg 864
Gly Trp Tyr Lys Ala His Asn Gln Glu Glu Gly Glu Tyr Tyr Asn Val
275 280 285

ccc att cca gaa gga gat gaa gaa ggc aac atg gaa ctc agg cag aag 912
Pro Ile Pro Glu Gly Asp Glu Glu Gly Asn Met Glu Leu Arg Gln Lys
290 295 300

ttt gag aaa gcc aag cta ggt cct gtt ggt aac aaa gtc atc agc cct 960
Phe Glu Lys Ala Lys Leu Gly Pro Val Gly Asn Lys Val Ile Ser Pro
305 310 315 320

tca gaa gac aga aag caa cca tcc aac aac ctg gac aga gtg aaa ctc 1008
Ser Glu Asp Arg Lys Gln Pro Ser Asn Asn Leu Asp Arg Val Lys Leu
325 330 335

aca gac ttc aac ttc ctc atg gtg ctg ggg aag ggg agt ttt ggg aag 1056
Thr Asp Phe Asn Phe Leu Met Val Leu Gly Lys Gly Ser Phe Gly Lys
340 345 350

gtg atg ctt gct gac agg aag gga acg gag gaa ctg tac gcc atc aag 1104
Val Met Leu Ala Asp Arg Lys Gly Thr Glu Glu Leu Tyr Ala Ile Lys
355 360 365

atc ctg aag aag gac gtg gtg atc cag gac gac gac gtg gag tgc acc 1152
Ile Leu Lys Lys Asp Val Val Ile Gln Asp Asp Asp Val Glu Cys Thr
370 375 380

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atg gtg gag aag cgc gtg ctg gcc ctg ctg gac aag ccg cca ttt ctg      1200
Met Val Glu Lys Arg Val Leu Ala Leu Leu Asp Lys Pro Pro Phe Leu
385                 390                 395                 400

aca cag ctg cac tcc tgc ttc cag aca gtg gac cgg ctg tac ttc gtc      1248
Thr Gln Leu His Ser Cys Phe Gln Thr Val Asp Arg Leu Tyr Phe Val
                405                 410                 415

atg gaa tac gtc aac ggc ggg gat ctt atg tac cac att cag caa gtc      1296
Met Glu Tyr Val Asn Gly Gly Asp Leu Met Tyr His Ile Gln Gln Val
            420                 425                 430

ggg aaa ttt aag gag cca caa gca gta ttc tac gca gcc gag atc tcc      1344
Gly Lys Phe Lys Glu Pro Gln Ala Val Phe Tyr Ala Ala Glu Ile Ser
        435                 440                 445

atc gga ctg ttc ttc ctt cat aaa aga ggg atc att tac agg gat ctg      1392
Ile Gly Leu Phe Phe Leu His Lys Arg Gly Ile Ile Tyr Arg Asp Leu
    450                 455                 460

aag ctg aac aat gtc atg ctg aac tca gaa ggg cac atc aaa atc gcc      1440
Lys Leu Asn Asn Val Met Leu Asn Ser Glu Gly His Ile Lys Ile Ala
465                 470                 475                 480

gac ttc ggg atg tgc aag gaa cac atg atg gat gga gtc acg acc agg      1488
Asp Phe Gly Met Cys Lys Glu His Met Met Asp Gly Val Thr Thr Arg
                485                 490                 495

acc ttc tgc gga act ccg gac tac att gcc cca gag ata atc gct tac      1536
Thr Phe Cys Gly Thr Pro Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr
            500                 505                 510

cag ccg tac ggg aag tct gta gat tgg tgg gcg tac ggt gtg ctg ctg      1584
Gln Pro Tyr Gly Lys Ser Val Asp Trp Trp Ala Tyr Gly Val Leu Leu
        515                 520                 525

tac gag atg cta gcc ggg cag cct ccg ttt gat ggt gaa gat gaa gat      1632
Tyr Glu Met Leu Ala Gly Gln Pro Pro Phe Asp Gly Glu Asp Glu Asp
    530                 535                 540

gaa ctg ttt cag tct ata atg gag cac aac gtg tcc tac ccc aaa tcc      1680
Glu Leu Phe Gln Ser Ile Met Glu His Asn Val Ser Tyr Pro Lys Ser
545                 550                 555                 560

ttg tcc aag gaa gcc gtc tcc atc tgc aaa gga ctt atg acc aaa cag      1728
Leu Ser Lys Glu Ala Val Ser Ile Cys Lys Gly Leu Met Thr Lys Gln
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cct gcc aag cga ctg ggc tgc ggg ccc gag gga gag agg gat gtc aga      1776
Pro Ala Lys Arg Leu Gly Cys Gly Pro Glu Gly Glu Arg Asp Val Arg
            580                 585                 590

gag cat gcc ttc ttc agg agg atc gac tgg gag aaa ctg gag aac agg      1824
Glu His Ala Phe Phe Arg Arg Ile Asp Trp Glu Lys Leu Glu Asn Arg
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Glu Ile Gln Pro Pro Phe Lys Pro Lys Val Cys Gly Lys Gly Ala Glu
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aac ttt gac aag ttc ttc acg cga gga cag cct gtc tta aca cca cca 1920
Asn Phe Asp Lys Phe Phe Thr Arg Gly Gln Pro Val Leu Thr Pro Pro
625 630 635 640

gat cag ctg gtc att gct aac ata gac caa tct gat ttt gaa ggg ttc 1968
Asp Gln Leu Val Ile Ala Asn Ile Asp Gln Ser Asp Phe Glu Gly Phe
645 650 655

tcg tat gtc aac ccc cag ttt gtg cac cca atc ttg caa agt gca gta 2016
Ser Tyr Val Asn Pro Gln Phe Val His Pro Ile Leu Gln Ser Ala Val
660 665 670

ggg cgc gcc atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca 2064
Gly Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
675 680 685

att ctt gtt gaa tta gat ggc gat gtt aat ggg caa aaa ttc tct gtt 2112
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val
690 695 700

agt gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa 2160
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
705 710 715 720

ttt att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc 2208
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
725 730 735

act act ctc act tat ggt gtt caa tgc ttt tct aga tac cca gat cat 2256
Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
740 745 750

atg aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta 2304
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
755 760 765

cag gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt 2352
Gln Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg
770 775 780

gct gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta 2400
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
785 790 795 800

aaa ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg 2448
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met
805 810 815

gaa tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca 2496
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro
820 825 830

aag aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat 2544
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp
835 840 845

gga agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc 2592
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
850 855 860

gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct 2640
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
865 870 875 880

gcc ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt 2688
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu
885 890 895

gag ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac 2736
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Lys Pro Gln Glu
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35 40 45

Phe Cys Ser His Cys Thr Asp Phe Ile Trp Gly Phe Gly Lys Gln Gly
50 55 60

Phe Gln Cys Gln Val Cys Cys Phe Val Val His Lys Arg Cys His Glu
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Phe Val Thr Phe Ser Cys Pro Gly Ala Asp Lys Gly Pro Asp Thr Asp
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Asp Pro Arg Ser Lys His Lys Phe Lys Ile His Thr Tyr Gly Ser Pro
100 105 110

Thr Phe Cys Asp His Cys Gly Ser Leu Leu Tyr Gly Leu Ile His Gln
115 120 125

Gly Met Lys Cys Asp Thr Cys Asp Met Asn Val His Asn Gln Cys Val
130 135 140

Ile Asn Asp Pro Ser Leu Cys Gly Met Asp His Thr Glu Lys Arg Gly
145 150 155 160

Arg Ile Tyr Leu Lys Ala Glu Val Thr Asp Glu Lys Leu His Val Thr
165 170 175

Val Arg Asp Ala Lys Asn Leu Ile Pro Met Asp Pro Asn Gly Leu Ser
180 185 190

Asp Pro Tyr Val Lys Leu Lys Leu Ile Pro Asp Pro Lys Asn Glu Ser
195 200 205

Lys Gln Lys Thr Lys Thr Ile Arg Ser Asn Leu Asn Pro Gln Trp Asn
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Glu Ser Phe Thr Phe Lys Leu Lys Pro Ser Asp Lys Asp Arg Arg Leu
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Ser Val Glu Ile Trp Asp Trp Asp Arg Thr Thr Arg Asn Asp Phe Met
245 250 255

Gly Ser Leu Ser Phe Gly Val Ser Glu Leu Met Lys Met Pro Ala Ser
260 265 270

Gly Trp Tyr Lys Ala His Asn Gln Glu Glu Gly Glu Tyr Tyr Asn Val
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Pro Ile Pro Glu Gly Asp Glu Glu Gly Asn Met Glu Leu Arg Gln Lys
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Phe Glu Lys Ala Lys Leu Gly Pro Val Gly Asn Lys Val Ile Ser Pro
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Ser Glu Asp Arg Lys Gln Pro Ser Asn Asn Leu Asp Arg Val Lys Leu
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Val Met Leu Ala Asp Arg Lys Gly Thr Glu Glu Leu Tyr Ala Ile Lys
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Ile Leu Lys Lys Asp Val Val Ile Gln Asp Asp Asp Val Glu Cys Thr
370 375 380

Met Val Glu Lys Arg Val Leu Ala Leu Leu Asp Lys Pro Pro Phe Leu
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Thr Gln Leu His Ser Cys Phe Gln Thr Val Asp Arg Leu Tyr Phe Val
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Met Glu Tyr Val Asn Gly Gly Asp Leu Met Tyr His Ile Gln Gln Val
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Gly Lys Phe Lys Glu Pro Gln Ala Val Phe Tyr Ala Ala Glu Ile Ser
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Ile Gly Leu Phe Phe Leu His Lys Arg Gly Ile Ile Tyr Arg Asp Leu
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Lys Leu Asn Asn Val Met Leu Asn Ser Glu Gly His Ile Lys Ile Ala
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Asp Phe Gly Met Cys Lys Glu His Met Met Asp Gly Val Thr Thr Arg
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Glu Leu Phe Gln Ser Ile Met Glu His Asn Val Ser Tyr Pro Lys Ser
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Pro Ala Lys Arg Leu Gly Cys Gly Pro Glu Gly Glu Arg Asp Val Arg
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Glu Ile Gln Pro Pro Phe Lys Pro Lys Val Cys Gly Lys Gly Ala Glu
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Asn Phe Asp Lys Phe Phe Thr Arg Gly Gln Pro Val Leu Thr Pro Pro
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Asp Gln Leu Val Ile Ala Asn Ile Asp Gln Ser Asp Phe Glu Gly Phe
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Ser Tyr Val Asn Pro Gln Phe Val His Pro Ile Leu Gln Ser Ala Val
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Gly Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
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Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val
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Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
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Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
                725   730   735

Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
            740   745   750

Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
        755   760   765

Gln Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg
770 775 780

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
785 790 795 800

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met
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Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro
820 825 830

Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp
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Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
850 855 860

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
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Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu
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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

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Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
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Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

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Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

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Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
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gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

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130 135 140

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165 170 175

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Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

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Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

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ggc ccg ggg gtc ccg ggg gag gtg gag atg gtg aag ggg cag ccg ttc      864
Gly Pro Gly Val Pro Gly Glu Val Glu Met Val Lys Gly Gln Pro Phe
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gac gtg ggc ccg cgc tac acg cag ttg cag tac atc ggc gag ggc gcg      912
Asp Val Gly Pro Arg Tyr Thr Gln Leu Gln Tyr Ile Gly Glu Gly Ala
    290                 295                 300

tac ggc atg gtc agc tcg gcc tat gac cac gtg cgc aag act cgc gtg      960
Tyr Gly Met Val Ser Ser Ala Tyr Asp His Val Arg Lys Thr Arg Val
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gcc atc aag aag atc agc ccc ttc gaa cat cag acc tac tgc cag cgc     1008
Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr Cys Gln Arg
                325                 330                 335

acg ctc cgg gag atc cag atc ctg ctg cgc ttc cgc cat gag aat gtc     1056
Thr Leu Arg Glu Ile Gln Ile Leu Leu Arg Phe Arg His Glu Asn Val
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Ile Gly Ile Arg Asp Ile Leu Arg Ala Ser Thr Leu Glu Ala Met Arg
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gat gtc tac att gtg cag gac ctg atg gag act gac ctg tac aag ttg     1152
Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu Tyr Lys Leu
    370                 375                 380

ctg aaa agc cag cag ctg agc aat gac cat atc tgc tac ttc ctc tac     1200
Leu Lys Ser Gln Gln Leu Ser Asn Asp His Ile Cys Tyr Phe Leu Tyr
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cag atc ctg cgg ggc ctc aag tac atc cac tcc gcc aac gtg ctc cac     1248
Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asn Val Leu His
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cga gat cta aag ccc tcc aac ctg ctc agc aac acc acc tgc gac ctt     1296
Arg Asp Leu Lys Pro Ser Asn Leu Leu Ser Asn Thr Thr Cys Asp Leu
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aag att tgt gat ttc ggc ctg gcc cgg att gcc gat cct gag cat gac     1344
Lys Ile Cys Asp Phe Gly Leu Ala Arg Ile Ala Asp Pro Glu His Asp
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cac acc ggc ttc ctg acg gag tat gtg gct acg cgc tgg tac cgg gcc     1392
His Thr Gly Phe Leu Thr Glu Tyr Val Ala Thr Arg Trp Tyr Arg Ala
    450                 455                 460
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Trp Ser Val Gly Cys Ile Leu Ala Glu Met Leu Ser Asn Arg Pro Ile
485 490 495

ttc cct ggc aag cac tac ctg gat cag ctc aac cac att ctg ggc atc 1536
Phe Pro Gly Lys His Tyr Leu Asp Gln Leu Asn His Ile Leu Gly Ile
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ctg ggc tcc cca tcc cag gag gac ctg aat tgt atc atc aac atg aag 1584
Leu Gly Ser Pro Ser Gln Glu Asp Leu Asn Cys Ile Ile Asn Met Lys
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gcc cga aac tac cta cag tct ctg ccc tcc aag acc aag gtg gct tgg 1632
Ala Arg Asn Tyr Leu Gln Ser Leu Pro Ser Lys Thr Lys Val Ala Trp
530 535 540

gcc aag ctt ttc ccc aag tca gac tcc aaa gcc ctt gac ctg ctg gac 1680
Ala Lys Leu Phe Pro Lys Ser Asp Ser Lys Ala Leu Asp Leu Leu Asp
545 550 555 560

cgg atg tta acc ttt aac ccc aat aaa cgg atc aca gtg gag gaa gcg 1728
Arg Met Leu Thr Phe Asn Pro Asn Lys Arg Ile Thr Val Glu Glu Ala
565 570 575

ctg gct cac ccc tac ctg gag cag tac tat gac ccg acg gat gag cca 1776
Leu Ala His Pro Tyr Leu Glu Gln Tyr Tyr Asp Pro Thr Asp Glu Pro
580 585 590

gtg gcc gag gag ccc ttc acc ttc gcc atg gag ctg gat gac cta cct 1824
Val Ala Glu Glu Pro Phe Thr Phe Ala Met Glu Leu Asp Asp Leu Pro
595 600 605

aag gag cgg ctg aag gag ctc atc ttc cag gag aca gca cgc ttc cag 1872
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35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ala Ala Ala
245 250 255

Ala Ala Gln Gly Gly Gly Gly Gly Glu Pro Arg Arg Thr Glu Gly Val
260 265 270

Gly Pro Gly Val Pro Gly Glu Val Glu Met Val Lys Gly Gln Pro Phe
275 280 285

Asp Val Gly Pro Arg Tyr Thr Gln Leu Gln Tyr Ile Gly Glu Gly Ala
290 295 300

Tyr Gly Met Val Ser Ser Ala Tyr Asp His Val Arg Lys Thr Arg Val
305 310 315 320

Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr Cys Gln Arg
325 330 335

Thr Leu Arg Glu Ile Gln Ile Leu Leu Arg Phe Arg His Glu Asn Val
340 345 350

Ile Gly Ile Arg Asp Ile Leu Arg Ala Ser Thr Leu Glu Ala Met Arg
355 360 365

Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu Tyr Lys Leu
370 375 380

Leu Lys Ser Gln Gln Leu Ser Asn Asp His Ile Cys Tyr Phe Leu Tyr
385 390 395 400

Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asn Val Leu His
405 410 415

Arg Asp Leu Lys Pro Ser Asn Leu Leu Ser Asn Thr Thr Cys Asp Leu
420 425 430

Lys Ile Cys Asp Phe Gly Leu Ala Arg Ile Ala Asp Pro Glu His Asp
435 440 445

His Thr Gly Phe Leu Thr Glu Tyr Val Ala Thr Arg Trp Tyr Arg Ala
450 455 460

Pro Glu Ile Met Leu Asn Ser Lys Gly Tyr Thr Lys Ser Ile Asp Ile
465 470 475 480

Trp Ser Val Gly Cys Ile Leu Ala Glu Met Leu Ser Asn Arg Pro Ile
485 490 495

Phe Pro Gly Lys His Tyr Leu Asp Gln Leu Asn His Ile Leu Gly Ile
500 505 510

Leu Gly Ser Pro Ser Gln Glu Asp Leu Asn Cys Ile Ile Asn Met Lys
515 520 525

Ala Arg Asn Tyr Leu Gln Ser Leu Pro Ser Lys Thr Lys Val Ala Trp
530 535 540

Ala Lys Leu Phe Pro Lys Ser Asp Ser Lys Ala Leu Asp Leu Leu Asp
545 550 555 560

Arg Met Leu Thr Phe Asn Pro Asn Lys Arg Ile Thr Val Glu Glu Ala
565 570 575

Leu Ala His Pro Tyr Leu Glu Gln Tyr Tyr Asp Pro Thr Asp Glu Pro
580 585 590

Val Ala Glu Glu Pro Phe Thr Phe Ala Met Glu Leu Asp Asp Leu Pro
595 600 605

Lys Glu Arg Leu Lys Glu Leu Ile Phe Gln Glu Thr Ala Arg Phe Gln
610 615 620

Pro Gly Val Leu Glu Ala Pro
625 630

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1 5 10 15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

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gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc      720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga gct caa gct tcg aat tcg acc atg tcg tcc atc      768
Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ser Ser Ile
                245                 250                 255

ttg cca ttc acg ccg cca gtt gtg aag aga ctg ctg gga tgg aag aag      816
Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu Gly Trp Lys Lys
            260                 265                 270

tca gct ggt ggg tct gga gga gca ggc gga gga gag cag aat ggg cag      864
Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu Gln Asn Gly Gln
        275                 280                 285

gaa gaa aag tgg tgt gag aaa gca gtg aaa agt ctg gtg aag aag cta      912
Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu Val Lys Lys Leu
    290                 295                 300

aag aaa aca gga cga tta gat gag ctt gag aaa gcc atc acc act caa      960
Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala Ile Thr Thr Gln
305                 310                 315                 320

aac tgt aat act aaa tgt gtt acc ata cca agc act tgc tct gaa att     1008
Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr Cys Ser Glu Ile
                325                 330                 335

tgg gga ctg agt aca cca aat acg ata gat cag tgg gat aca aca ggc     1056
Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp Asp Thr Thr Gly
            340                 345                 350

ctt tac agc ttc tct gaa caa acc agg tct ctt gat ggt cgt ctc cag     1104
Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp Gly Arg Leu Gln
        355                 360                 365

gta tcc cat cga aaa gga ttg cca cat gtt ata tat tgc cga tta tgg     1152
Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr Cys Arg Leu Trp
    370                 375                 380

cgc tgg cct gat ctt cac agt cat cat gaa ctc aag gca att gaa aac     1200
Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys Ala Ile Glu Asn
385                 390                 395                 400

tgc gaa tat gct ttt aat ctt aaa aag gat gaa gta tgt gta aac cct     1248
Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val Cys Val Asn Pro
                405                 410                 415

tac cac tat cag aga gtt gag aca cca gtt ttg cct cca gta tta gtg     1296
Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro Pro Val Leu Val
            420                 425                 430

ccc cga cac acc gag atc cta aca gaa ctt ccg cct ctg gat gac tat     1344
Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro Leu Asp Asp Tyr
        435                 440                 445
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act cac tcc att cca gaa aac act aac ttc cca gca gga att gag cca 1392
Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala Gly Ile Glu Pro
450 455 460

cag agt aat tat att cca gaa acg cca cct cct gga tat atc agt gaa 1440
Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly Tyr Ile Ser Glu
465 470 475 480

gat gga gaa aca agt gac caa cag ttg aat caa agt atg gac aca ggc 1488
Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser Met Asp Thr Gly
485 490 495

tct cca gca gaa cta tct cct act act ctt tcc cct gtt aat cat agc 1536
Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro Val Asn His Ser
500 505 510

ttg gat tta cag cca gtt act tac tca gaa cct gca ttt tgg tgt tca 1584
Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala Phe Trp Cys Ser
515 520 525

ata gca tat tat gaa tta aat cag agg gtt gga gaa acc ttc cat gca 1632
Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu Thr Phe His Ala
530 535 540

tca cag ccc tca ctc act gta gat ggc ttt aca gac cca tca aat tca 1680
Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp Pro Ser Asn Ser
545 550 555 560

gag agg ttc tgc tta ggt tta ctc tcc aat gtt aac cga aat gcc acg 1728
Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn Arg Asn Ala Thr
565 570 575

gta gaa atg aca aga agg cat ata gga aga gga gtg cgc tta tac tac 1776
Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val Arg Leu Tyr Tyr
580 585 590

ata ggt ggg gaa gtt ttt gct gag tgc cta agt gat agt gca atc ttt 1824
Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp Ser Ala Ile Phe
595 600 605

gtg cag agc ccc aat tgt aat cag aga tat ggc tgg cac cct gca aca 1872
Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp His Pro Ala Thr
610 615 620

gtg tgt aaa att cca cca ggc tgt aat ctg aag atc ttc aac aac cag 1920
Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile Phe Asn Asn Gln
625 630 635 640

gaa ttt gct gct ctt ctg gct cag tct gtt aat cag ggt ttt gaa gcc 1968
Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln Gly Phe Glu Ala
645 650 655

gtc tat cag cta act aga atg tgc acc ata aga atg agt ttt gtg aaa 2016
Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met Ser Phe Val Lys
660 665 670

ggg tgg gga gca gaa tac cga agg cag acg gta aca agt act cct tgc 2064
Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr Ser Thr Pro Cys
675 680 685

tgg att gaa ctt cat ctg aat gga cct cta cag tgg ttg gac aaa gta 2112
Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp Leu Asp Lys Val
690 695 700

tta act cag atg gga tcc cct tca gtg cgt tgc tca agc atg tca taa 2160
Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser Ser Met Ser
705 710 715

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<213> Aequorea victoria and human

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ser Ser Ile
245 250 255

Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu Gly Trp Lys Lys
260 265 270

Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu Gln Asn Gly Gln
275 280 285

Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu Val Lys Lys Leu
290 295 300

Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala Ile Thr Thr Gln
305 310 315 320

Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr Cys Ser Glu Ile
325 330 335

Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp Asp Thr Thr Gly
340 345 350

Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp Gly Arg Leu Gln
355 360 365

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Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr Cys Arg Leu Trp
    370                 375                 380

Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys Ala Ile Glu Asn
385                 390                 395                 400

Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val Cys Val Asn Pro
                405                 410                 415

Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro Pro Val Leu Val
            420                 425                 430

Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro Leu Asp Asp Tyr
        435                 440                 445

Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala Gly Ile Glu Pro
    450                 455                 460

Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly Tyr Ile Ser Glu
465                 470                 475                 480

Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser Met Asp Thr Gly
                485                 490                 495

Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro Val Asn His Ser
            500                 505                 510

Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala Phe Trp Cys Ser
        515                 520                 525

Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu Thr Phe His Ala
    530                 535                 540

Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp Pro Ser Asn Ser
545                 550                 555                 560

Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn Arg Asn Ala Thr
                565                 570                 575

Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val Arg Leu Tyr Tyr
            580                 585                 590
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Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp Ser Ala Ile Phe
595 600 605

Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp His Pro Ala Thr
610 615 620

Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile Phe Asn Asn Gln
625 630 635 640

Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln Gly Phe Glu Ala
645 650 655

Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met Ser Phe Val Lys
660 665 670

Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr Ser Thr Pro Cys
675 680 685

Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp Leu Asp Lys Val
690 695 700

Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser Ser Met Ser
705 710 715

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Met Ser Ser Ile Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu
1 5 10 15

gga tgg aag aag tca gct ggt ggg tct gga gga gca ggc gga gga gag 96
Gly Trp Lys Lys Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu
20 25 30

cag aat ggg cag gaa gaa aag tgg tgt gag aaa gca gtg aaa agt ctg 144
Gln Asn Gly Gln Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu
35 40 45

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gtg aag aag cta aag aaa aca gga cga tta gat gag ctt gag aaa gcc       192
Val Lys Lys Leu Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala
    50                  55                  60

atc acc act caa aac tgt aat act aaa tgt gtt acc ata cca agc act       240
Ile Thr Thr Gln Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr
65                  70                  75                  80

tgc tct gaa att tgg gga ctg agt aca cca aat acg ata gat cag tgg       288
Cys Ser Glu Ile Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp
                85                  90                  95

gat aca aca ggc ctt tac agc ttc tct gaa caa acc agg tct ctt gat       336
Asp Thr Thr Gly Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp
            100                 105                 110

ggt cgt ctc cag gta tcc cat cga aaa gga ttg cca cat gtt ata tat       384
Gly Arg Leu Gln Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr
        115                 120                 125

tgc cga tta tgg cgc tgg cct gat ctt cac agt cat cat gaa ctc aag       432
Cys Arg Leu Trp Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys
    130                 135                 140

gca att gaa aac tgc gaa tat gct ttt aat ctt aaa aag gat gaa gta       480
Ala Ile Glu Asn Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val
145                 150                 155                 160

tgt gta aac cct tac cac tat cag aga gtt gag aca cca gtt ttg cct       528
Cys Val Asn Pro Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro
                165                 170                 175

cca gta tta gtg ccc cga cac acc gag atc cta aca gaa ctt ccg cct       576
Pro Val Leu Val Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro
            180                 185                 190

ctg gat gac tat act cac tcc att cca gaa aac act aac ttc cca gca       624
Leu Asp Asp Tyr Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala
        195                 200                 205

gga att gag cca cag agt aat tat att cca gaa acg cca cct cct gga       672
Gly Ile Glu Pro Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly
    210                 215                 220

tat atc agt gaa gat gga gaa aca agt gac caa cag ttg aat caa agt       720
Tyr Ile Ser Glu Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser
225                 230                 235                 240

atg gac aca ggc tct cca gca gaa cta tct cct act act ctt tcc cct       768
Met Asp Thr Gly Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro
                245                 250                 255

gtt aat cat agc ttg gat tta cag cca gtt act tac tca gaa cct gca       816
Val Asn His Ser Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala
            260                 265                 270
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ttt tgg tgt tca ata gca tat tat gaa tta aat cag agg gtt gga gaa 864
Phe Trp Cys Ser Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu
275 280 285

acc ttc cat gca tca cag ccc tca ctc act gta gat ggc ttt aca gac 912
Thr Phe His Ala Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp
290 295 300

cca tca aat tca gag agg ttc tgc tta ggt tta ctc tcc aat gtt aac 960
Pro Ser Asn Ser Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn
305 310 315 320

cga aat gcc acg gta gaa atg aca aga agg cat ata gga aga gga gtg 1008
Arg Asn Ala Thr Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val
325 330 335

cgc tta tac tac ata ggt ggg gaa gtt ttt gct gag tgc cta agt gat 1056
Arg Leu Tyr Tyr Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp
340 345 350

agt gca atc ttt gtg cag agc ccc aat tgt aat cag aga tat ggc tgg 1104
Ser Ala Ile Phe Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp
355 360 365

cac cct gca aca gtg tgt aaa att cca cca ggc tgt aat ctg aag atc 1152
His Pro Ala Thr Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile
370 375 380

ttc aac aac cag gaa ttt gct gct ctt ctg gct cag tct gtt aat cag 1200
Phe Asn Asn Gln Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln
385 390 395 400

ggt ttt gaa gcc gtc tat cag cta act aga atg tgc acc ata aga atg 1248
Gly Phe Glu Ala Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met
405 410 415

agt ttt gtg aaa ggg tgg gga gca gaa tac cga agg cag acg gta aca 1296
Ser Phe Val Lys Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr
420 425 430

agt act cct tgc tgg att gaa ctt cat ctg aat gga cct cta cag tgg 1344
Ser Thr Pro Cys Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp
435 440 445

ttg gac aaa gta tta act cag atg gga tcc cct tca gtg cgt tgc tca 1392
Leu Asp Lys Val Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser
450 455 460

agc atg tca tgg gta ccg cgg gcc cgg gat cca ccg gtc gcc acc atg 1440
Ser Met Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met
465 470 475 480

gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc 1488
Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
485 490 495

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gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag      1536
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            500                 505                 510

ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc      1584
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        515                 520                 525

acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg      1632
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
    530                 535                 540

acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag cag      1680
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
545                 550                 555                 560

cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc      1728
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                565                 570                 575

acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg      1776
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            580                 585                 590

aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc      1824
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        595                 600                 605

gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac      1872
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    610                 615                 620

tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac ggc      1920
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
625                 630                 635                 640

atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg      1968
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                645                 650                 655

cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc      2016
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            660                 665                 670

gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc      2064
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        675                 680                 685

aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg      2112
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    690                 695                 700

acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa          2157
Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
705                 710                 715
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<212> PRT
<213> Aequorea victoria and human

<400> 10

Met Ser Ser Ile Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu
1   5   10   15

Gly Trp Lys Lys Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu
            20   25   30

Gln Asn Gly Gln Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu
        35   40   45

Val Lys Lys Leu Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala
    50   55   60

Ile Thr Thr Gln Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr
65   70   75   80

Cys Ser Glu Ile Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp
                85   90   95

Asp Thr Thr Gly Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp
            100   105   110

Gly Arg Leu Gln Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr
        115   120   125

Cys Arg Leu Trp Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys
    130   135   140

Ala Ile Glu Asn Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val
145   150   155   160

Cys Val Asn Pro Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro
                165   170   175

Pro Val Leu Val Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro
            180   185   190

Leu Asp Asp Tyr Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala
195 200 205

Gly Ile Glu Pro Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly
210 215 220

Tyr Ile Ser Glu Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser
225 230 235 240

Met Asp Thr Gly Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro
245 250 255

Val Asn His Ser Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala
260 265 270

Phe Trp Cys Ser Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu
275 280 285

Thr Phe His Ala Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp
290 295 300

Pro Ser Asn Ser Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn
305 310 315 320

Arg Asn Ala Thr Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val
325 330 335

Arg Leu Tyr Tyr Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp
340 345 350

Ser Ala Ile Phe Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp
355 360 365

His Pro Ala Thr Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile
370 375 380

Phe Asn Asn Gln Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln
385 390 395 400

Gly Phe Glu Ala Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met
405 410 415

Ser Phe Val Lys Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr
420 425 430

Ser Thr Pro Cys Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp
435 440 445

Leu Asp Lys Val Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser
450 455 460

Ser Met Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met
465 470 475 480

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
485 490 495

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
500 505 510

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
515 520 525

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
530 535 540

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
545 550 555 560

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
565 570 575

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
580 585 590

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
595 600 605

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
610 615 620

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
625 630 635 640

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Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                645                 650                 655

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            660                 665                 670

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        675                 680                 685

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    690                 695                 700

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
705                 710                 715


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atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg       48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1               5                   10                  15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc       96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc      144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc      192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag      240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag      288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95
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```
cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag        336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc        384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac        432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac        480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc        528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc        576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg        624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc        672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc        720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga gct caa gct tcc atg agc gag acg gtc atc atg        768
Gly Leu Arg Ser Arg Ala Gln Ala Ser Met Ser Glu Thr Val Ile Met
                245                 250                 255

agc gag acg gtc atc tgt tcc agc cgg gcc act gtg atg ctt tat gat        816
Ser Glu Thr Val Ile Cys Ser Ser Arg Ala Thr Val Met Leu Tyr Asp
            260                 265                 270

gat ggc aac aag cga tgg ctc cct gct ggc acg ggt ccc cag gcc ttc        864
Asp Gly Asn Lys Arg Trp Leu Pro Ala Gly Thr Gly Pro Gln Ala Phe
        275                 280                 285

agc cgc gtc cag atc tac cac aac ccc acg gcc aat tcc ttt cgc gtc        912
Ser Arg Val Gln Ile Tyr His Asn Pro Thr Ala Asn Ser Phe Arg Val
    290                 295                 300

gtg ggc cgg aag atg cag ccc gac cag cag gtg gtc atc aac tgt gcc        960
Val Gly Arg Lys Met Gln Pro Asp Gln Gln Val Val Ile Asn Cys Ala
305                 310                 315                 320
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atc gtc cgg ggt gtc aag tat aac cag gcc acc ccc aac ttc cat cag      1008
Ile Val Arg Gly Val Lys Tyr Asn Gln Ala Thr Pro Asn Phe His Gln
                325                 330                 335

tgg cgc gac gct cgc cag gtc tgg ggc ctc aac ttc ggc agc aag gag      1056
Trp Arg Asp Ala Arg Gln Val Trp Gly Leu Asn Phe Gly Ser Lys Glu
            340                 345                 350

gat gcg gcc cag ttt gcc gcc ggc atg gcc agt gcc cta gag gcg ttg      1104
Asp Ala Ala Gln Phe Ala Ala Gly Met Ala Ser Ala Leu Glu Ala Leu
        355                 360                 365

gaa gga ggt ggg ccc cct cca ccc cca gca ctt ccc acc tgg tcg gtc      1152
Glu Gly Gly Gly Pro Pro Pro Pro Pro Ala Leu Pro Thr Trp Ser Val
    370                 375                 380

ccg aac ggc ccc tcc ccg gag gag gtg gag cag cag aaa agg cag cag      1200
Pro Asn Gly Pro Ser Pro Glu Glu Val Glu Gln Gln Lys Arg Gln Gln
385                 390                 395                 400

ccc ggc ccg tcg gag cac ata gag cgc cgg gtc tcc aat gca gga ggc      1248
Pro Gly Pro Ser Glu His Ile Glu Arg Arg Val Ser Asn Ala Gly Gly
                405                 410                 415

cca cct gct ccc ccc gct ggg ggt cca ccc cca cca cca gga cct ccc      1296
Pro Pro Ala Pro Pro Ala Gly Gly Pro Pro Pro Pro Pro Gly Pro Pro
            420                 425                 430

cct cct cca ggt ccc ccc cca ccc cca ggt ttg ccc cct tcg ggg gtc      1344
Pro Pro Pro Gly Pro Pro Pro Pro Pro Gly Leu Pro Pro Ser Gly Val
        435                 440                 445

cca gct gca gcg cac gga gca ggg gga gga cca ccc cct gca ccc cct      1392
Pro Ala Ala Ala His Gly Ala Gly Gly Gly Pro Pro Pro Ala Pro Pro
    450                 455                 460

ctc ccg gca gca cag ggc cct ggt ggt ggg gga gct ggg gcc cca ggc      1440
Leu Pro Ala Ala Gln Gly Pro Gly Gly Gly Gly Ala Gly Ala Pro Gly
465                 470                 475                 480

ctg gcc gca gct att gct gga gcc aaa ctc agg aaa gtc agc aag cag      1488
Leu Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Lys Gln
                485                 490                 495

gag gag gcc tca ggg ggg ccc aca gcc ccc aaa gct gag agt ggt cga      1536
Glu Glu Ala Ser Gly Gly Pro Thr Ala Pro Lys Ala Glu Ser Gly Arg
            500                 505                 510

agc gga ggt ggg gga ctc atg gaa gag atg aac gcc atg ctg gcc cgg      1584
Ser Gly Gly Gly Gly Leu Met Glu Glu Met Asn Ala Met Leu Ala Arg
        515                 520                 525

aga agg aaa gcc acg caa gtt ggg gag aaa acc ccc aag gat gaa tct      1632
Arg Arg Lys Ala Thr Gln Val Gly Glu Lys Thr Pro Lys Asp Glu Ser
    530                 535                 540
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gcc aat cag gag gag cca gag gcc aga gtc ccg gcc cag agt gaa tct 1680
Ala Asn Gln Glu Glu Pro Glu Ala Arg Val Pro Ala Gln Ser Glu Ser
545 550 555 560

gtg cgg aga ccc tgg gag aag aac agc aca acc ttg cca agg atg aag 1728
Val Arg Arg Pro Trp Glu Lys Asn Ser Thr Thr Leu Pro Arg Met Lys
565 570 575

tcg tct tct tcg gtg acc act tcc gag acc caa ccc tgc acg ccc agc 1776
Ser Ser Ser Ser Val Thr Thr Ser Glu Thr Gln Pro Cys Thr Pro Ser
580 585 590

tcc agt gat tac tcg gac cta cag agg gtg aaa cag gag ctt ctg gaa 1824
Ser Ser Asp Tyr Ser Asp Leu Gln Arg Val Lys Gln Glu Leu Leu Glu
595 600 605

gag gtg aag aag gaa ttg cag aaa gtg aaa gag gaa atc att gaa gcc 1872
Glu Val Lys Lys Glu Leu Gln Lys Val Lys Glu Glu Ile Ile Glu Ala
610 615 620

ttc gtc cag gag ctg agg aag cgg ggt tct ccc tga 1908
Phe Val Gln Glu Leu Arg Lys Arg Gly Ser Pro
625 630 635

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<212> PRT
<213> Aequorea victoria and human

<400> 12

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Gln Ala Ser Met Ser Glu Thr Val Ile Met
245 250 255

Ser Glu Thr Val Ile Cys Ser Ser Arg Ala Thr Val Met Leu Tyr Asp
260 265 270

Asp Gly Asn Lys Arg Trp Leu Pro Ala Gly Thr Gly Pro Gln Ala Phe
275 280 285

Ser Arg Val Gln Ile Tyr His Asn Pro Thr Ala Asn Ser Phe Arg Val
290 295 300

Val Gly Arg Lys Met Gln Pro Asp Gln Gln Val Val Ile Asn Cys Ala
305 310 315 320

Ile Val Arg Gly Val Lys Tyr Asn Gln Ala Thr Pro Asn Phe His Gln
325 330 335

Trp Arg Asp Ala Arg Gln Val Trp Gly Leu Asn Phe Gly Ser Lys Glu
340 345 350

Asp Ala Ala Gln Phe Ala Ala Gly Met Ala Ser Ala Leu Glu Ala Leu
355 360 365

Glu Gly Gly Gly Pro Pro Pro Pro Pro Ala Leu Pro Thr Trp Ser Val
370 375 380

Pro Asn Gly Pro Ser Pro Glu Glu Val Glu Gln Gln Lys Arg Gln Gln
385 390 395 400

Pro Gly Pro Ser Glu His Ile Glu Arg Arg Val Ser Asn Ala Gly Gly
405 410 415

Pro Pro Ala Pro Pro Ala Gly Gly Pro Pro Pro Pro Pro Gly Pro Pro
420 425 430

Pro Pro Pro Gly Pro Pro Pro Pro Pro Gly Leu Pro Pro Ser Gly Val
435 440 445

Pro Ala Ala Ala His Gly Ala Gly Gly Gly Pro Pro Pro Ala Pro Pro
450 455 460

Leu Pro Ala Ala Gln Gly Pro Gly Gly Gly Gly Ala Gly Ala Pro Gly
465 470 475 480

Leu Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Lys Gln
485 490 495

Glu Glu Ala Ser Gly Gly Pro Thr Ala Pro Lys Ala Glu Ser Gly Arg
500 505 510

Ser Gly Gly Gly Gly Leu Met Glu Glu Met Asn Ala Met Leu Ala Arg
515 520 525

Arg Arg Lys Ala Thr Gln Val Gly Glu Lys Thr Pro Lys Asp Glu Ser
530 535 540

Ala Asn Gln Glu Glu Pro Glu Ala Arg Val Pro Ala Gln Ser Glu Ser
545 550 555 560

Val Arg Arg Pro Trp Glu Lys Asn Ser Thr Thr Leu Pro Arg Met Lys
565 570 575

Ser Ser Ser Ser Val Thr Thr Ser Glu Thr Gln Pro Cys Thr Pro Ser
580 585 590

Ser Ser Asp Tyr Ser Asp Leu Gln Arg Val Lys Gln Glu Leu Leu Glu
595 600 605

Glu Val Lys Lys Glu Leu Gln Lys Val Lys Glu Glu Ile Ile Glu Ala
610 615 620

Phe Val Gln Glu Leu Arg Lys Arg Gly Ser Pro
625 630 635

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<211> 2394
<212> DNA
<213> Aequorea victoria and human

<220>
<221> CDS
<222> (1)..(2394)
<223>

<400> 13
atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg 48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

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cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag      288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag      336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc      384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac      432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac      480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc      528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc      576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg      624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc      672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc      720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga gcc atg gac gaa ctg ttc ccc ctc atc ttc ccg      768
Gly Leu Arg Ser Arg Ala Met Asp Glu Leu Phe Pro Leu Ile Phe Pro
                245                 250                 255

gca gag cca gcc cag gcc tct ggc ccc tat gtg gag atc att gag cag      816
Ala Glu Pro Ala Gln Ala Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln
            260                 265                 270

ccc aag cag cgg ggc atg cgc ttc cgc tac aag tgc gag ggg cgc tcc      864
Pro Lys Gln Arg Gly Met Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser
        275                 280                 285

gcg ggc agc atc cca ggc gag agg agc aca gat acc acc aag acc cac      912
Ala Gly Ser Ile Pro Gly Glu Arg Ser Thr Asp Thr Thr Lys Thr His
    290                 295                 300
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ccc acc atc aag atc aat ggc tac aca gga cca ggg aca gtg cgc atc      960
Pro Thr Ile Lys Ile Asn Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile
305                 310                 315                 320

tcc ctg gtc acc aag gac cct cct cac cgg cct cac ccc cac gag ctt     1008
Ser Leu Val Thr Lys Asp Pro Pro His Arg Pro His Pro His Glu Leu
                325                 330                 335

gta gga aag gac tgc cgg gat ggc ttc tat gag gct gag ctc tgc ccg     1056
Val Gly Lys Asp Cys Arg Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro
            340                 345                 350

gac cgc tgc atc cac agt ttc cag aac ctg gga atc cag tgt gtg aag     1104
Asp Arg Cys Ile His Ser Phe Gln Asn Leu Gly Ile Gln Cys Val Lys
        355                 360                 365

aag cgg gac ctg gag cag gct atc agt cag cgc atc cag acc aac aac     1152
Lys Arg Asp Leu Glu Gln Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn
    370                 375                 380

aac ccc ttc caa gtt cct ata gaa gag cag cgt ggg gac tac gac ctg     1200
Asn Pro Phe Gln Val Pro Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu
385                 390                 395                 400

aat gct gtg cgg ctc tgc ttc cag gtg aca gtg cgg gac cca tca ggc     1248
Asn Ala Val Arg Leu Cys Phe Gln Val Thr Val Arg Asp Pro Ser Gly
                405                 410                 415

agg ccc ctc cgc ctg ccg cct gtc ctt cct cat ccc atc ttt gac aat     1296
Arg Pro Leu Arg Leu Pro Pro Val Leu Pro His Pro Ile Phe Asp Asn
            420                 425                 430

cgt gcc ccc aac act gcc gag ctc aag atc tgc cga gtg aac cga aac     1344
Arg Ala Pro Asn Thr Ala Glu Leu Lys Ile Cys Arg Val Asn Arg Asn
        435                 440                 445

tct ggc agc tgc ctc ggt ggg gat gag atc ttc cta ctg tgt gac aag     1392
Ser Gly Ser Cys Leu Gly Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys
    450                 455                 460

gtg cag aaa gag gac att gag gtg tat ttc acg gga cca ggc tgg gag     1440
Val Gln Lys Glu Asp Ile Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu
465                 470                 475                 480

gcc cga ggc tcc ttt tcg caa gct gat gtg cac cga caa gtg gcc att     1488
Ala Arg Gly Ser Phe Ser Gln Ala Asp Val His Arg Gln Val Ala Ile
                485                 490                 495

gtg ttc cgg acc cct ccc tac gca gac ccc agc ctg cag gct cct gtg     1536
Val Phe Arg Thr Pro Pro Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val
            500                 505                 510

cgt gtc tcc atg cag ctg cgg cgg cct tcc gac cgg gag ctc agt gag     1584
Arg Val Ser Met Gln Leu Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu
        515                 520                 525
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ccc atg gaa ttc cag tac ctg cca gat aca gac gat cgt cac cgg att      1632
Pro Met Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg Ile
    530                 535                 540

gag gag aaa cgt aaa agg aca tat gag acc ttc aag agc atc atg aag      1680
Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys
545                 550                 555                 560

aag agt cct ttc agc gga ccc acc gac ccc cgg cct cca cct cga cgc      1728
Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg
                565                 570                 575

att gct gtg cct tcc cgc agc tca gct tct gtc ccc aag cca gca ccc      1776
Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala Pro
            580                 585                 590

cag ccc tat ccc ttt acg tca tcc ctg agc acc atc aac tat gat gag      1824
Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu
        595                 600                 605

ttt ccc acc atg gtg ttt cct tct ggg cag atc agc cag gcc tcg gcc      1872
Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala
    610                 615                 620

ttg gcc ccg gcc cct ccc caa gtc ctg ccc cag gct cca gcc cct gcc      1920
Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala
625                 630                 635                 640

cct gct cca gcc atg gta tca gct ctg gcc cag gcc cca gcc cct gtc      1968
Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val
                645                 650                 655

cca gtc cta gcc cca ggc cct cct cag gct gtg gcc cca cct gcc ccc      2016
Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro
            660                 665                 670

aag ccc acc cag gct ggg gaa gga acg ctg tca gag gcc ctg ctg cag      2064
Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln
        675                 680                 685

ctg cag ttt gat gat gaa gac ctg ggg gcc ttg ctt ggc aac agc aca      2112
Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr
    690                 695                 700

gac cca gct gtg ttc aca gac ctg gca tcc gtc gac aac tcc gag ttt      2160
Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe
705                 710                 715                 720

cag cag ctg ctg aac cag ggc ata cct gtg gcc ccc cac aca act gag      2208
Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu
                725                 730                 735

ccc atg ctg atg gag tac cct gag gct ata act cgc cta gtg aca ggg      2256
Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly
            740                 745                 750
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gcc cag agg ccc ccc gac cca gct cct gct cca ctg ggg gcc ccg ggg 2304
Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly
755 760 765

ctc ccc aat ggc ctc ctt tca gga gat gaa gac ttc tcc tcc att gcg 2352
Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala
770 775 780

gac atg gac ttc tca gcc ctg ctg agt cag atc agc tcc taa 2394
Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
785 790 795

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

```
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

Gly Leu Arg Ser Arg Ala Met Asp Glu Leu Phe Pro Leu Ile Phe Pro
                245                 250                 255

Ala Glu Pro Ala Gln Ala Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln
            260                 265                 270

Pro Lys Gln Arg Gly Met Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser
        275                 280                 285

Ala Gly Ser Ile Pro Gly Glu Arg Ser Thr Asp Thr Thr Lys Thr His
    290                 295                 300

Pro Thr Ile Lys Ile Asn Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile
305                 310                 315                 320

Ser Leu Val Thr Lys Asp Pro Pro His Arg Pro His Pro His Glu Leu
                325                 330                 335

Val Gly Lys Asp Cys Arg Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro
            340                 345                 350

Asp Arg Cys Ile His Ser Phe Gln Asn Leu Gly Ile Gln Cys Val Lys
        355                 360                 365
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Lys Arg Asp Leu Glu Gln Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn
    370                 375                 380

Asn Pro Phe Gln Val Pro Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu
385                 390                 395                 400

Asn Ala Val Arg Leu Cys Phe Gln Val Thr Val Arg Asp Pro Ser Gly
                405                 410                 415

Arg Pro Leu Arg Leu Pro Pro Val Leu Pro His Pro Ile Phe Asp Asn
            420                 425                 430

Arg Ala Pro Asn Thr Ala Glu Leu Lys Ile Cys Arg Val Asn Arg Asn
        435                 440                 445

Ser Gly Ser Cys Leu Gly Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys
    450                 455                 460

Val Gln Lys Glu Asp Ile Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu
465                 470                 475                 480

Ala Arg Gly Ser Phe Ser Gln Ala Asp Val His Arg Gln Val Ala Ile
                485                 490                 495

Val Phe Arg Thr Pro Pro Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val
            500                 505                 510

Arg Val Ser Met Gln Leu Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu
        515                 520                 525

Pro Met Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg Ile
    530                 535                 540

Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys
545                 550                 555                 560

Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg
                565                 570                 575

Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala Pro
            580                 585                 590
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Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu
595 600 605

Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala
610 615 620

Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala
625 630 635 640

Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val
645 650 655

Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro
660 665 670

Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln
675 680 685

Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr
690 695 700

Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe
705 710 715 720

Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu
725 730 735

Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly
740 745 750

Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly
755 760 765

Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala
770 775 780

Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
785 790 795

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atg gac gaa ctg ttc ccc ctc atc ttc ccg gca gag cca gcc cag gcc       48
Met Asp Glu Leu Phe Pro Leu Ile Phe Pro Ala Glu Pro Ala Gln Ala
1               5                   10                  15

tct ggc ccc tat gtg gag atc att gag cag ccc aag cag cgg ggc atg       96
Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
            20                  25                  30

cgc ttc cgc tac aag tgc gag ggg cgc tcc gcg ggc agc atc cca ggc      144
Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
        35                  40                  45

gag agg agc aca gat acc acc aag acc cac ccc acc atc aag atc aat      192
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
    50                  55                  60

ggc tac aca gga cca ggg aca gtg cgc atc tcc ctg gtc acc aag gac      240
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65                  70                  75                  80

cct cct cac cgg cct cac ccc cac gag ctt gta gga aag gac tgc cgg      288
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
                85                  90                  95

gat ggc ttc tat gag gct gag ctc tgc ccg gac cgc tgc atc cac agt      336
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
            100                 105                 110

ttc cag aac ctg gga atc cag tgt gtg aag aag cgg gac ctg gag cag      384
Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
        115                 120                 125

gct atc agt cag cgc atc cag acc aac aac aac ccc ttc caa gtt cct      432
Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
    130                 135                 140

ata gaa gag cag cgt ggg gac tac gac ctg aat gct gtg cgg ctc tgc      480
Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145                 150                 155                 160

ttc cag gtg aca gtg cgg gac cca tca ggc agg ccc ctc cgc ctg ccg      528
Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
                165                 170                 175

cct gtc ctt cct cat ccc atc ttt gac aat cgt gcc ccc aac act gcc      576
Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
            180                 185                 190
```

gag ctc aag atc tgc cga gtg aac cga aac tct ggc agc tgc ctc ggt 624
Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
195 200 205

ggg gat gag atc ttc cta ctg tgt gac aag gtg cag aaa gag gac att 672
Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
210 215 220

gag gtg tat ttc acg gga cca ggc tgg gag gcc cga ggc tcc ttt tcg 720
Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225 230 235 240

caa gct gat gtg cac cga caa gtg gcc att gtg ttc cgg acc cct ccc 768
Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
245 250 255

tac gca gac ccc agc ctg cag gct cct gtg cgt gtc tcc atg cag ctg 816
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
260 265 270

cgg cgg cct tcc gac cgg gag ctc agt gag ccc atg gaa ttc cag tac 864
Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
275 280 285

ctg cca gat aca gac gat cgt cac cgg att gag gag aaa cgt aaa agg 912
Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
290 295 300

aca tat gag acc ttc aag agc atc atg aag aag agt cct ttc agc gga 960
Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305 310 315 320

ccc acc gac ccc cgg cct cca cct cga cgc att gct gtg cct tcc cgc 1008
Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
325 330 335

agc tca gct tct gtc ccc aag cca gca ccc cag ccc tat ccc ttt acg 1056
Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
340 345 350

tca tcc ctg agc acc atc aac tat gat gag ttt ccc acc atg gtg ttt 1104
Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
355 360 365

cct tct ggg cag atc agc cag gcc tcg gcc ttg gcc ccg gcc cct ccc 1152
Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
370 375 380

caa gtc ctg ccc cag gct cca gcc cct gcc cct gct cca gcc atg gta 1200
Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385 390 395 400

tca gct ctg gcc cag gcc cca gcc cct gtc cca gtc cta gcc cca ggc 1248
Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
405 410 415

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cct cct cag gct gtg gcc cca cct gcc ccc aag ccc acc cag gct ggg      1296
Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
            420                 425                 430

gaa gga acg ctg tca gag gcc ctg ctg cag ctg cag ttt gat gat gaa      1344
Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
        435                 440                 445

gac ctg ggg gcc ttg ctt ggc aac agc aca gac cca gct gtg ttc aca      1392
Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
    450                 455                 460

gac ctg gca tcc gtc gac aac tcc gag ttt cag cag ctg ctg aac cag      1440
Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465                 470                 475                 480

ggc ata cct gtg gcc ccc cac aca act gag ccc atg ctg atg gag tac      1488
Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
                485                 490                 495

cct gag gct ata act cgc cta gtg aca ggg gcc cag agg ccc ccc gac      1536
Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
            500                 505                 510

cca gct cct gct cca ctg ggg gcc ccg ggg ctc ccc aat ggc ctc ctt      1584
Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
        515                 520                 525

tca gga gat gaa gac ttc tcc tcc att gcg gac atg gac ttc tca gcc      1632
Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
    530                 535                 540

ctg ctg agt cag atc agc tcc ttg gat cca ccg gtc gcc acc atg gtg      1680
Leu Leu Ser Gln Ile Ser Ser Leu Asp Pro Pro Val Ala Thr Met Val
545                 550                 555                 560

agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag      1728
Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
                565                 570                 575

ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc      1776
Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly
            580                 585                 590

gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc acc      1824
Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
        595                 600                 605

acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc      1872
Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr
    610                 615                 620

tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag cag cac      1920
Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His
625                 630                 635                 640
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gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc 1968
Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr
645 650 655

atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg aag 2016
Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys
660 665 670

ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac 2064
Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp
675 680 685

ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac 2112
Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr
690 695 700

aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac ggc atc 2160
Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
705 710 715 720

aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag 2208
Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln
725 730 735

ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg 2256
Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
740 745 750

ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc aaa 2304
Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys
755 760 765

gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc 2352
Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
770 775 780

gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa 2394
Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
785 790 795

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Met Asp Glu Leu Phe Pro Leu Ile Phe Pro Ala Glu Pro Ala Gln Ala
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Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
20 25 30

Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
35 40 45

Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
50 55 60

Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65 70 75 80

Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
85 90 95

Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
100 105 110

Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
115 120 125

Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
130 135 140

Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145 150 155 160

Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
165 170 175

Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
180 185 190

Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
195 200 205

Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
210 215 220

Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225 230 235 240

Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
245 250 255

```
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
            260                 265                 270

Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
        275                 280                 285

Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
    290                 295                 300

Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305                 310                 315                 320

Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
                325                 330                 335

Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
            340                 345                 350

Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
        355                 360                 365

Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
    370                 375                 380

Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385                 390                 395                 400

Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
                405                 410                 415

Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
            420                 425                 430

Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
        435                 440                 445

Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
    450                 455                 460

Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465                 470                 475                 480
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Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
                485   490   495

Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
            500   505   510

Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
        515   520   525

Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
    530   535   540

Leu Leu Ser Gln Ile Ser Ser Leu Asp Pro Pro Val Ala Thr Met Val
545   550   555   560

Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
                565   570   575

Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly
            580   585   590

Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
        595   600   605

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr
    610   615   620

Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His
625   630   635   640

Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr
                645   650   655

Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys
            660   665   670

Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp
        675   680   685

Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr
    690   695   700

```
Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
705                 710                 715                 720

Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln
                725                 730                 735

Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
            740                 745                 750

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys
        755                 760                 765

Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
    770                 775                 780

Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
785                 790                 795


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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1               5                   10                  15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc       96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc      144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc      192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag      240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80
```

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cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag      288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag      336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc      384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac      432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac      480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc      528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc      576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg      624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc      672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc      720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga ggc aag atg gct gac ccg gct gcg ggg ccg ccg      768
Gly Leu Arg Ser Arg Gly Lys Met Ala Asp Pro Ala Ala Gly Pro Pro
                245                 250                 255

ccg agc gag ggc gag gag agc acc gtg cgc ttc gcc cgc aaa ggc gcc      816
Pro Ser Glu Gly Glu Glu Ser Thr Val Arg Phe Ala Arg Lys Gly Ala
            260                 265                 270

ctc cgg cag aag aac gtg cat gag gtc aag aac cac aaa ttc acc gcc      864
Leu Arg Gln Lys Asn Val His Glu Val Lys Asn His Lys Phe Thr Ala
        275                 280                 285

cgc ttc ttc aag cag ccc acc ttc tgc agc cac tgc acc gac ttc atc      912
Arg Phe Phe Lys Gln Pro Thr Phe Cys Ser His Cys Thr Asp Phe Ile
    290                 295                 300
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tgg ggc ttc ggg aag cag gga ttc cag tgc caa gtt tgc tgc ttt gtg 960
Trp Gly Phe Gly Lys Gln Gly Phe Gln Cys Gln Val Cys Cys Phe Val
305 310 315 320

gtg cac aag cgg tgc cat gaa ttt gtc aca ttc tcc tgc cct ggc gct 1008
Val His Lys Arg Cys His Glu Phe Val Thr Phe Ser Cys Pro Gly Ala
325 330 335

gac aag ggt cca gcc tcc gat gac ccc cgc agc aaa cac aag ttt aag 1056
Asp Lys Gly Pro Ala Ser Asp Asp Pro Arg Ser Lys His Lys Phe Lys
340 345 350

atc cac acg tac tcc agc ccc acg ttt tgt gac cac tgt ggg tca ctg 1104
Ile His Thr Tyr Ser Ser Pro Thr Phe Cys Asp His Cys Gly Ser Leu
355 360 365

ctg tat gga ctc atc cac cag ggg atg aaa tgt gac acc tgc atg atg 1152
Leu Tyr Gly Leu Ile His Gln Gly Met Lys Cys Asp Thr Cys Met Met
370 375 380

aat gtg cac aag cgc tgc gtg atg aat gtt ccc agc ctg tgt ggc acg 1200
Asn Val His Lys Arg Cys Val Met Asn Val Pro Ser Leu Cys Gly Thr
385 390 395 400

gac cac acg gag cgc cgc ggc cgc atc tac atc cag gcc cac atc gac 1248
Asp His Thr Glu Arg Arg Gly Arg Ile Tyr Ile Gln Ala His Ile Asp
405 410 415

agg gac gtc ctc att gtc ctc gta aga gat gct aaa aac ctt gta cct 1296
Arg Asp Val Leu Ile Val Leu Val Arg Asp Ala Lys Asn Leu Val Pro
420 425 430

atg gac ccc aat ggc ctg tca gat ccc tac gta aaa ctg aaa ctg att 1344
Met Asp Pro Asn Gly Leu Ser Asp Pro Tyr Val Lys Leu Lys Leu Ile
435 440 445

ccc gat ccc aaa agt gag agc aaa cag aag acc aaa acc atc aaa tgc 1392
Pro Asp Pro Lys Ser Glu Ser Lys Gln Lys Thr Lys Thr Ile Lys Cys
450 455 460

tcc ctc aac cct gag tgg aat gag aca ttt aga ttt cag ctg aaa gaa 1440
Ser Leu Asn Pro Glu Trp Asn Glu Thr Phe Arg Phe Gln Leu Lys Glu
465 470 475 480

tcg gac aaa gac aga aga ctg tca gta gag att tgg gat tgg gat ttg 1488
Ser Asp Lys Asp Arg Arg Leu Ser Val Glu Ile Trp Asp Trp Asp Leu
485 490 495

acc agc agg aat gac ttc atg gga tct ttg tcc ttt ggg att tct gaa 1536
Thr Ser Arg Asn Asp Phe Met Gly Ser Leu Ser Phe Gly Ile Ser Glu
500 505 510

ctt cag aag gcc agt gtt gat ggc tgg ttt aag tta ctg agc cag gag 1584
Leu Gln Lys Ala Ser Val Asp Gly Trp Phe Lys Leu Leu Ser Gln Glu
515 520 525

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gaa ggc gag tac ttc aat gtg cct gtg cca cca gaa gga agt gag gcc      1632
Glu Gly Glu Tyr Phe Asn Val Pro Val Pro Pro Glu Gly Ser Glu Ala
    530                 535                 540

aat gaa gaa ctg cgg cag aaa ttt gag agg gcc aag atc agt cag gga      1680
Asn Glu Glu Leu Arg Gln Lys Phe Glu Arg Ala Lys Ile Ser Gln Gly
545                 550                 555                 560

acc aag gtc ccg gaa gaa aag acg acc aac act gtc tcc aaa ttt gac      1728
Thr Lys Val Pro Glu Glu Lys Thr Thr Asn Thr Val Ser Lys Phe Asp
                565                 570                 575

aac aat ggc aac aga gac cgg atg aaa ctg acc gat ttt aac ttc cta      1776
Asn Asn Gly Asn Arg Asp Arg Met Lys Leu Thr Asp Phe Asn Phe Leu
            580                 585                 590

atg gtg ctg ggg aaa ggc agc ttt ggc aag gtc atg ctt tca gaa cga      1824
Met Val Leu Gly Lys Gly Ser Phe Gly Lys Val Met Leu Ser Glu Arg
        595                 600                 605

aaa ggc aca gat gag ctc tat gct gtg aag atc ctg aag aag gac gtt      1872
Lys Gly Thr Asp Glu Leu Tyr Ala Val Lys Ile Leu Lys Lys Asp Val
    610                 615                 620

gtg atc caa gat gat gac gtg gag tgc act atg gtg gag aag cgg gtg      1920
Val Ile Gln Asp Asp Asp Val Glu Cys Thr Met Val Glu Lys Arg Val
625                 630                 635                 640

ttg gcc ctg cct ggg aag ccg ccc ttc ctg acc cag ctc cac tcc tgc      1968
Leu Ala Leu Pro Gly Lys Pro Pro Phe Leu Thr Gln Leu His Ser Cys
                645                 650                 655

ttc cag acc atg gac cgc ctg tac ttt gtg atg gag tac gtg aat ggg      2016
Phe Gln Thr Met Asp Arg Leu Tyr Phe Val Met Glu Tyr Val Asn Gly
            660                 665                 670

ggc gac ctc atg tat cac atc cag caa gtc ggc cgg ttc aag gag ccc      2064
Gly Asp Leu Met Tyr His Ile Gln Gln Val Gly Arg Phe Lys Glu Pro
        675                 680                 685

cat gct gta ttt tac gct gca gaa att gcc atc ggt ctg ttc ttc tta      2112
His Ala Val Phe Tyr Ala Ala Glu Ile Ala Ile Gly Leu Phe Phe Leu
    690                 695                 700

cag agt aag ggc atc att tac cgt gac cta aaa ctt gac aac gtg atg      2160
Gln Ser Lys Gly Ile Ile Tyr Arg Asp Leu Lys Leu Asp Asn Val Met
705                 710                 715                 720

ctc gat tct gag gga cac atc aag att gcc gat ttt ggc atg tgt aag      2208
Leu Asp Ser Glu Gly His Ile Lys Ile Ala Asp Phe Gly Met Cys Lys
                725                 730                 735

gaa aac atc tgg gat ggg gtg aca acc aag aca ttc tgt ggc act cca      2256
Glu Asn Ile Trp Asp Gly Val Thr Thr Lys Thr Phe Cys Gly Thr Pro
            740                 745                 750
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gac tac atc gcc ccc gag ata att gct tat cag ccc tat ggg aag tcc 2304
Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr Gln Pro Tyr Gly Lys Ser
755 760 765

gtg gat tgg tgg gca ttt gga gtc ctg ctg tat gaa atg ttg gct ggg 2352
Val Asp Trp Trp Ala Phe Gly Val Leu Leu Tyr Glu Met Leu Ala Gly
770 775 780

cag gca ccc ttt gaa ggg gag gat gaa gat gaa ctc ttc caa tcc atc 2400
Gln Ala Pro Phe Glu Gly Glu Asp Glu Asp Glu Leu Phe Gln Ser Ile
785 790 795 800

atg gaa cac aac gta gcc tat ccc aag tct atg tcc aag gaa gct gtg 2448
Met Glu His Asn Val Ala Tyr Pro Lys Ser Met Ser Lys Glu Ala Val
805 810 815

gcc atc tgc aaa ggg ctg atg acc aaa cac cca ggc aaa cgt ctg ggt 2496
Ala Ile Cys Lys Gly Leu Met Thr Lys His Pro Gly Lys Arg Leu Gly
820 825 830

tgt gga cct gaa ggc gaa cgt gat atc aaa gag cat gca ttt ttc cgg 2544
Cys Gly Pro Glu Gly Glu Arg Asp Ile Lys Glu His Ala Phe Phe Arg
835 840 845

tat att gat tgg gag aaa ctt gaa cgc aaa gag atc cag ccc cct tat 2592
Tyr Ile Asp Trp Glu Lys Leu Glu Arg Lys Glu Ile Gln Pro Pro Tyr
850 855 860

aag cca aaa gct aga gac aag aga gac acc tcc aac ttc gac aaa gag 2640
Lys Pro Lys Ala Arg Asp Lys Arg Asp Thr Ser Asn Phe Asp Lys Glu
865 870 875 880

ttc acc aga cag cct gtg gaa ctg acc ccc act gat aaa ctc ttc atc 2688
Phe Thr Arg Gln Pro Val Glu Leu Thr Pro Thr Asp Lys Leu Phe Ile
885 890 895

atg aac ttg gac caa aat gaa ttt gct ggc ttc tct tat act aac cca 2736
Met Asn Leu Asp Gln Asn Glu Phe Ala Gly Phe Ser Tyr Thr Asn Pro
900 905 910

gag ttt gtc att aat gtg tag 2757
Glu Phe Val Ile Asn Val
915

<210> 18
<211> 918
<212> PRT
<213> Aequorea victoria and human

<400> 18

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240
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Gly Leu Arg Ser Arg Gly Lys Met Ala Asp Pro Ala Ala Gly Pro Pro
245 250 255

Pro Ser Glu Gly Glu Glu Ser Thr Val Arg Phe Ala Arg Lys Gly Ala
260 265 270

Leu Arg Gln Lys Asn Val His Glu Val Lys Asn His Lys Phe Thr Ala
275 280 285

Arg Phe Phe Lys Gln Pro Thr Phe Cys Ser His Cys Thr Asp Phe Ile
290 295 300

Trp Gly Phe Gly Lys Gln Gly Phe Gln Cys Gln Val Cys Cys Phe Val
305 310 315 320

Val His Lys Arg Cys His Glu Phe Val Thr Phe Ser Cys Pro Gly Ala
325 330 335

Asp Lys Gly Pro Ala Ser Asp Asp Pro Arg Ser Lys His Lys Phe Lys
340 345 350

Ile His Thr Tyr Ser Ser Pro Thr Phe Cys Asp His Cys Gly Ser Leu
355 360 365

Leu Tyr Gly Leu Ile His Gln Gly Met Lys Cys Asp Thr Cys Met Met
370 375 380

Asn Val His Lys Arg Cys Val Met Asn Val Pro Ser Leu Cys Gly Thr
385 390 395 400

Asp His Thr Glu Arg Arg Gly Arg Ile Tyr Ile Gln Ala His Ile Asp
405 410 415

Arg Asp Val Leu Ile Val Leu Val Arg Asp Ala Lys Asn Leu Val Pro
420 425 430

Met Asp Pro Asn Gly Leu Ser Asp Pro Tyr Val Lys Leu Lys Leu Ile
435 440 445

Pro Asp Pro Lys Ser Glu Ser Lys Gln Lys Thr Lys Thr Ile Lys Cys
450 455 460

Ser Leu Asn Pro Glu Trp Asn Glu Thr Phe Arg Phe Gln Leu Lys Glu
465 470 475 480

Ser Asp Lys Asp Arg Arg Leu Ser Val Glu Ile Trp Asp Trp Asp Leu
485 490 495

Thr Ser Arg Asn Asp Phe Met Gly Ser Leu Ser Phe Gly Ile Ser Glu
500 505 510

Leu Gln Lys Ala Ser Val Asp Gly Trp Phe Lys Leu Leu Ser Gln Glu
515 520 525

Glu Gly Glu Tyr Phe Asn Val Pro Val Pro Pro Glu Gly Ser Glu Ala
530 535 540

Asn Glu Glu Leu Arg Gln Lys Phe Glu Arg Ala Lys Ile Ser Gln Gly
545 550 555 560

Thr Lys Val Pro Glu Glu Lys Thr Thr Asn Thr Val Ser Lys Phe Asp
565 570 575

Asn Asn Gly Asn Arg Asp Arg Met Lys Leu Thr Asp Phe Asn Phe Leu
580 585 590

Met Val Leu Gly Lys Gly Ser Phe Gly Lys Val Met Leu Ser Glu Arg
595 600 605

Lys Gly Thr Asp Glu Leu Tyr Ala Val Lys Ile Leu Lys Lys Asp Val
610 615 620

Val Ile Gln Asp Asp Asp Val Glu Cys Thr Met Val Glu Lys Arg Val
625 630 635 640

Leu Ala Leu Pro Gly Lys Pro Pro Phe Leu Thr Gln Leu His Ser Cys
645 650 655

Phe Gln Thr Met Asp Arg Leu Tyr Phe Val Met Glu Tyr Val Asn Gly
660 665 670

Gly Asp Leu Met Tyr His Ile Gln Gln Val Gly Arg Phe Lys Glu Pro
675 680 685

His Ala Val Phe Tyr Ala Ala Glu Ile Ala Ile Gly Leu Phe Phe Leu
690 695 700

Gln Ser Lys Gly Ile Ile Tyr Arg Asp Leu Lys Leu Asp Asn Val Met
705 710 715 720

Leu Asp Ser Glu Gly His Ile Lys Ile Ala Asp Phe Gly Met Cys Lys
725 730 735

Glu Asn Ile Trp Asp Gly Val Thr Thr Lys Thr Phe Cys Gly Thr Pro
740 745 750

Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr Gln Pro Tyr Gly Lys Ser
755 760 765

Val Asp Trp Trp Ala Phe Gly Val Leu Leu Tyr Glu Met Leu Ala Gly
770 775 780

Gln Ala Pro Phe Glu Gly Glu Asp Glu Asp Glu Leu Phe Gln Ser Ile
785 790 795 800

Met Glu His Asn Val Ala Tyr Pro Lys Ser Met Ser Lys Glu Ala Val
805 810 815

Ala Ile Cys Lys Gly Leu Met Thr Lys His Pro Gly Lys Arg Leu Gly
820 825 830

Cys Gly Pro Glu Gly Glu Arg Asp Ile Lys Glu His Ala Phe Phe Arg
835 840 845

Tyr Ile Asp Trp Glu Lys Leu Glu Arg Lys Glu Ile Gln Pro Pro Tyr
850 855 860

Lys Pro Lys Ala Arg Asp Lys Arg Asp Thr Ser Asn Phe Asp Lys Glu
865 870 875 880

Phe Thr Arg Gln Pro Val Glu Leu Thr Pro Thr Asp Lys Leu Phe Ile
885 890 895

Met Asn Leu Asp Gln Asn Glu Phe Ala Gly Phe Ser Tyr Thr Asn Pro
900 905 910

Glu Phe Val Ile Asn Val
        915

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<220>
<223> Primer used to construct the PKAc-F64L-S65T-GFP fusion

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<210> 20
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKAc-F64L-S65T-GFP fusion

<400> 20
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<210> 21
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKAc-F64L-S65T-GFP fusion

<400> 21
ttggacacaa gctttggaca cggcgcgcca tgagtaaagg agaagaactt ttc   53

<210> 22
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKAc-F64L-S65T-GFP fusion

<400> 22
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<210> 23
<211> 55
<212> DNA
<213> Artificial sequence

<220>
<223> Primer used to construct the PKC-GFP fusion

<400> 23
ttggacacaa gctttggaca ccctcaggat atggctgacg tttacccggc caacg   55

<210> 24
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKC-GFP fusion

<400> 24
gtcatcttct cgagtctttc aggcgcgccc tactgcactt tgcaagattg ggtgc   55

<210> 25
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKC-GFP fusion

<400> 25
ttggacacaa gctttggaca cggcgcgcca tgagtaaagg agaagaactt ttc   53

<210> 26
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer used to construct the PKC-GFP fusion

<400> 26
gtcatcttct cgagtcttac tcctgaggtt tgtatagttc atccatgcca tgt   53

<210> 27
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer targeted to Homo sapiens

<400> 27
tagaattcaa ccatggcggc ggcggcggcg   30

<210> 28
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<212> DNA
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<223> Primer targeted to Homo sapiens

<400> 28
taggatccct agggggcctc cagcactcc 29

<210> 29
<211> 33
<212> DNA
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<400> 29
gtgaattcga ccatgtcgtc catcttgcca ttc 33

<210> 30
<211> 32
<212> DNA
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<220>
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<400> 30
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<210> 31
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<210> 32
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<210> 33
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<400> 33
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<210> 36
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<400> 36
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<400> 37
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<210> 38
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<400> 38
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<210> 39
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<400> 39
gtctcgaggc aagatggctg accc 24

<210> 40
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<220>
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<400> 40
gtggatccct acacattaat gacaaactct ggg 33

SEQUENCE LISTING

<110> BioImage A/S

<120> AN IMPROVED METHOD FOR EXTRACTING
QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A
CELLULAR RESPONSE.

<130> 22129PC1

<160> 18

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<210> 1
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<400> 1

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 1               5                  10                  15

aaa gag ttc cta gcc aaa gcc aag gaa gat ttc ctg aaa aaa tgg gaa       96
Lys Glu Phe Leu Ala Lys Ala Lys Glu Asp Phe Leu Lys Lys Trp Glu
            20                  25                  30

gac ccc tct cag aat aca gcc cag ttg gat cag ttt gat aga atc aag      144
Asp Pro Ser Gln Asn Thr Ala Gln Leu Asp Gln Phe Asp Arg Ile Lys
        35                  40                  45

acc ctt ggc acc ggc tcc ttt ggg cga gtg atg ctg gtg aag cac aag      192
Thr Leu Gly Thr Gly Ser Phe Gly Arg Val Met Leu Val Lys His Lys
    50                  55                  60
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gag agt ggg aac cac tac gcc atg aag atc tta gac aag cag aag gtg      240
Glu Ser Gly Asn His Tyr Ala Met Lys Ile Leu Asp Lys Gln Lys Val
 65                  70                  75                  80

gtg aag cta aag cag atc gag cac act ctg aat gag aag cgc atc ctg      288
Val Lys Leu Lys Gln Ile Glu His Thr Leu Asn Glu Lys Arg Ile Leu
                 85                  90                  95

cag gcc gtc aac ttc ccg ttc ctg gtc aaa ctt gaa ttc tcc ttc aag      336
Gln Ala Val Asn Phe Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys
            100                 105                 110

gac aac tca aac ctg tac atg gtc atg gag tat gta gct ggt ggc gag      384
Asp Asn Ser Asn Leu Tyr Met Val Met Glu Tyr Val Ala Gly Gly Glu
        115                 120                 125

atg ttc tcc cac cta cgg cgg att gga agg ttc agc gag ccc cat gcc      432
Met Phe Ser His Leu Arg Arg Ile Gly Arg Phe Ser Glu Pro His Ala
    130                 135                 140

cgt ttc tac gcg gcg cag atc gtc ctg acc ttt gag tat ctg cac tcc      480
Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser
145                 150                 155                 160

ctg gac ctc atc tac cgg gac ctg aag ccc gag aat ctt ctc atc gac      528
Leu Asp Leu Ile Tyr Arg Asp Leu Lys Pro Glu Asn Leu Leu Ile Asp
                165                 170                 175

cag cag ggc tat att cag gtg aca gac ttc ggt ttt gcc aag cgt gtg      576
Gln Gln Gly Tyr Ile Gln Val Thr Asp Phe Gly Phe Ala Lys Arg Val
            180                 185                 190

aaa ggc cgt act tgg acc ttg tgt ggg acc cct gag tac ttg gcc ccc      624
Lys Gly Arg Thr Trp Thr Leu Cys Gly Thr Pro Glu Tyr Leu Ala Pro
        195                 200                 205

gag att atc ctg agc aaa ggc tac aac aag gct gtg gac tgg tgg gct      672
Glu Ile Ile Leu Ser Lys Gly Tyr Asn Lys Ala Val Asp Trp Trp Ala
    210                 215                 220
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ctc gga gtc ctc atc tac gag atg gct gct ggt tac cca ccc ttc ttc 720
Leu Gly Val Leu Ile Tyr Glu Met Ala Ala Gly Tyr Pro Pro Phe Phe
225 230 235 240

gct gac cag cct atc cag atc tat gag aaa atc gtc tct ggg aag gtg 768
Ala Asp Gln Pro Ile Gln Ile Tyr Glu Lys Ile Val Ser Gly Lys Val
245 250 255

cgg ttc cca tcc cac ttc agc tct gac ttg aag gac ctg ctg cgg aac 816
Arg Phe Pro Ser His Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn
260 265 270

ctt ctg caa gtg gat cta acc aag cgc ttt gga aac ctc aag gac ggg 864
Leu Leu Gln Val Asp Leu Thr Lys Arg Phe Gly Asn Leu Lys Asp Gly
275 280 285

gtc aat gac atc aag aac cac aag tgg ttt gcc acg act gac tgg att 912
Val Asn Asp Ile Lys Asn His Lys Trp Phe Ala Thr Thr Asp Trp Ile
290 295 300

gcc atc tat cag aga aag gtg gaa gct ccc ttc ata cca aag ttt aaa 960
Ala Ile Tyr Gln Arg Lys Val Glu Ala Pro Phe Ile Pro Lys Phe Lys
305 310 315 320

ggc cct ggg gac acg agt aac ttt gac gac tat gag gag gaa gag atc 1008
Gly Pro Gly Asp Thr Ser Asn Phe Asp Asp Tyr Glu Glu Glu Glu Ile
325 330 335

cgg gtc tcc atc aat gag aag tgt ggc aag gag ttt act gag ttt ggg 1056
Arg Val Ser Ile Asn Glu Lys Cys Gly Lys Glu Phe Thr Glu Phe Gly
340 345 350

cgc gcc atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att 1104
Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
355 360 365

ctt gtt gaa tta gat ggc gat gtt aat ggg caa aaa ttc tct gtt agt 1152
Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser
370 375 380

gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 1200
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
385 390 395 400

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 1248
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
405 410 415

act ctc act tat ggt gtt caa tgc ttt tct aga tac cca gat cat atg 1296
Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
420 425 430

aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag 1344
Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
435 440 445

gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct 1392
Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
450 455 460

gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa 1440
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
465 470 475 480

ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa 1488
Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu
485 490 495

tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca aag 1536
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys
500 505 510

aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat gga 1584
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly
515 520 525

agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc gat 1632
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
530 535 540

ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct gcc 1680
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
545 550 555 560

ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag 1728
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu
565 570 575

ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa 1776
Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
580 585 590

cct cag gag taa 1788
Pro Gln Glu *
595

<210> 2
<211> 595
<212> PRT
<213> Aequorea victoria and mouse

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20 25 30
Asp Pro Ser Gln Asn Thr Ala Gln Leu Asp Gln Phe Asp Arg Ile Lys
35 40 45
Thr Leu Gly Thr Gly Ser Phe Gly Arg Val Met Leu Val Lys His Lys
50 55 60
Glu Ser Gly Asn His Tyr Ala Met Lys Ile Leu Asp Lys Gln Lys Val
65 70 75 80
Val Lys Leu Lys Gln Ile Glu His Thr Leu Asn Glu Lys Arg Ile Leu
85 90 95
Gln Ala Val Asn Phe Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys
100 105 110
Asp Asn Ser Asn Leu Tyr Met Val Met Glu Tyr Val Ala Gly Gly Glu
115 120 125

Met Phe Ser His Leu Arg Arg Ile Gly Arg Phe Ser Glu Pro His Ala
130 135 140

Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser
145 150 155 160

Leu Asp Leu Ile Tyr Arg Asp Leu Lys Pro Glu Asn Leu Leu Ile Asp
165 170 175

Gln Gln Gly Tyr Ile Gln Val Thr Asp Phe Gly Phe Ala Lys Arg Val
180 185 190

Lys Gly Arg Thr Trp Thr Leu Cys Gly Thr Pro Glu Tyr Leu Ala Pro
195 200 205

Glu Ile Ile Leu Ser Lys Gly Tyr Asn Lys Ala Val Asp Trp Trp Ala
210 215 220

Leu Gly Val Leu Ile Tyr Glu Met Ala Ala Gly Tyr Pro Pro Phe Phe
225 230 235 240

Ala Asp Gln Pro Ile Gln Ile Tyr Glu Lys Ile Val Ser Gly Lys Val
245 250 255

Arg Phe Pro Ser His Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn
260 265 270

Leu Leu Gln Val Asp Leu Thr Lys Arg Phe Gly Asn Leu Lys Asp Gly
275 280 285

Val Asn Asp Ile Lys Asn His Lys Trp Phe Ala Thr Thr Asp Trp Ile
290 295 300

Ala Ile Tyr Gln Arg Lys Val Glu Ala Pro Phe Ile Pro Lys Phe Lys
305 310 315 320

Gly Pro Gly Asp Thr Ser Asn Phe Asp Asp Tyr Glu Glu Glu Glu Ile
325 330 335

Arg Val Ser Ile Asn Glu Lys Cys Gly Lys Glu Phe Thr Glu Phe Gly
340 345 350

Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
355 360 365

Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser
370 375 380

Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
385 390 395 400

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
405 410 415

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
420 425 430

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
435 440 445

Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
450 455 460
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
465 470 475 480
Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu
485 490 495
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys
500 505 510
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly
515 520 525
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
530 535 540
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
545 550 555 560
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu
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Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
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Pro Gln Glu
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Ala Asn Arg Phe Ala Arg Lys Gly Ala Leu Arg Gln Lys Asn Val His
20 25 30

gag gtg aaa gac cac aaa ttc atc gcc cgc ttc ttc aag caa ccc acc 144

Glu Val Lys Asp His Lys Phe Ile Ala Arg Phe Phe Lys Gln Pro Thr
35 40 45

ttc tgc agc cac tgc acc gac ttc atc tgg ggg ttt ggg aaa caa ggc 192
Phe Cys Ser His Cys Thr Asp Phe Ile Trp Gly Phe Gly Lys Gln Gly
50 55 60

ttc cag tgc caa gtt tgc tgt ttt gtg gtt cat aag agg tgc cat gag 240
Phe Gln Cys Gln Val Cys Cys Phe Val Val His Lys Arg Cys His Glu
65 70 75 80

ttc gtt acg ttc tct tgt ccg ggt gcg gat aag gga cct gac act gac 288
Phe Val Thr Phe Ser Cys Pro Gly Ala Asp Lys Gly Pro Asp Thr Asp
85 90 95

gac ccc agg agc aag cac aag ttc aaa atc cac aca tac gga agc cct 336
Asp Pro Arg Ser Lys His Lys Phe Lys Ile His Thr Tyr Gly Ser Pro
100 105 110

acc ttc tgt gat cac tgt ggg tcc ctg ctc tat gga ctt atc cac caa 384
Thr Phe Cys Asp His Cys Gly Ser Leu Leu Tyr Gly Leu Ile His Gln
115 120 125

ggg atg aaa tgt gac acc tgc gac atg aat gtt cac aac cag tgt gtg 432
Gly Met Lys Cys Asp Thr Cys Asp Met Asn Val His Asn Gln Cys Val
130 135 140

atc aat gac cct agc ctc tgc gga atg gat cac aca gag aag agg ggg 480
Ile Asn Asp Pro Ser Leu Cys Gly Met Asp His Thr Glu Lys Arg Gly
145 150 155 160

cgg att tat ctg aag gct gag gtc act gat gaa aag ctc cac gtc acg 528
Arg Ile Tyr Leu Lys Ala Glu Val Thr Asp Glu Lys Leu His Val Thr
165 170 175

gta cga gat gca aaa aat cta atc cct atg gat cca aat ggg ctt tcg 576
Val Arg Asp Ala Lys Asn Leu Ile Pro Met Asp Pro Asn Gly Leu Ser
180 185 190

gat cct tat gtg aag ctg aaa cta atc cct gac ccc aag aat gag agc 624

Asp Pro Tyr Val Lys Leu Lys Leu Ile Pro Asp Pro Lys Asn Glu Ser
195 200 205

aaa cag aaa acc aaa acc atc cgc tcc aac ctg aat cct cag tgg aat 672
Lys Gln Lys Thr Lys Thr Ile Arg Ser Asn Leu Asn Pro Gln Trp Asn
210 215 220

gag tcc ttc acg ttc aaa tta aaa cct tca gac aaa gac cgg cga ctg 720
Glu Ser Phe Thr Phe Lys Leu Lys Pro Ser Asp Lys Asp Arg Arg Leu
225 230 235 240

tct gta gaa atc tgg gac tgg gat cgg acg act cgg aat gac ttc atg 768
Ser Val Glu Ile Trp Asp Trp Asp Arg Thr Thr Arg Asn Asp Phe Met
245 250 255

gga tcc ctt tcc ttt ggt gtc tca gag cta atg aag atg ccg gcc agt 816
Gly Ser Leu Ser Phe Gly Val Ser Glu Leu Met Lys Met Pro Ala Ser
260 265 270

gga tgg tat aaa gct cac aac caa gaa gag ggc gaa tat tac aac gtg 864
Gly Trp Tyr Lys Ala His Asn Gln Glu Glu Gly Glu Tyr Tyr Asn Val
275 280 285

ccc att cca gaa gga gat gaa gaa ggc aac atg gaa ctc agg cag aag 912
Pro Ile Pro Glu Gly Asp Glu Glu Gly Asn Met Glu Leu Arg Gln Lys
290 295 300

ttt gag aaa gcc aag cta ggt cct gtt ggt aac aaa gtc atc agc cct 960
Phe Glu Lys Ala Lys Leu Gly Pro Val Gly Asn Lys Val Ile Ser Pro
305 310 315 320

tca gaa gac aga aag caa cca tcc aac aac ctg gac aga gtg aaa ctc 1008
Ser Glu Asp Arg Lys Gln Pro Ser Asn Asn Leu Asp Arg Val Lys Leu
325 330 335

aca gac ttc aac ttc ctc atg gtg ctg ggg aag ggg agt ttt ggg aag 1056
Thr Asp Phe Asn Phe Leu Met Val Leu Gly Lys Gly Ser Phe Gly Lys
340 345 350

gtg atg ctt gct gac agg aag gga acg gag gaa ctg tac gcc atc aag 1104

Val Met Leu Ala Asp Arg Lys Gly Thr Glu Glu Leu Tyr Ala Ile Lys
355 360 365

atc ctg aag aag gac gtg gtg atc cag gac gac gac gtg gag tgc acc 1152
Ile Leu Lys Lys Asp Val Val Ile Gln Asp Asp Asp Val Glu Cys Thr
370 375 380

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Met Val Glu Lys Arg Val Leu Ala Leu Leu Asp Lys Pro Pro Phe Leu
385 390 395 400

aca cag ctg cac tcc tgc ttc cag aca gtg gac cgg ctg tac ttc gtc 1248
Thr Gln Leu His Ser Cys Phe Gln Thr Val Asp Arg Leu Tyr Phe Val
405 410 415

atg gaa tac gtc aac ggc ggg gat ctt atg tac cac att cag caa gtc 1296
Met Glu Tyr Val Asn Gly Gly Asp Leu Met Tyr His Ile Gln Gln Val
420 425 430

ggg aaa ttt aag gag cca caa gca gta ttc tac gca gcc gag atc tcc 1344
Gly Lys Phe Lys Glu Pro Gln Ala Val Phe Tyr Ala Ala Glu Ile Ser
435 440 445

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Ile Gly Leu Phe Phe Leu His Lys Arg Gly Ile Ile Tyr Arg Asp Leu
450 455 460

aag ctg aac aat gtc atg ctg aac tca gaa ggg cac atc aaa atc gcc 1440
Lys Leu Asn Asn Val Met Leu Asn Ser Glu Gly His Ile Lys Ile Ala
465 470 475 480

gac ttc ggg atg tgc aag gaa cac atg atg gat gga gtc acg acc agg 1488
Asp Phe Gly Met Cys Lys Glu His Met Met Asp Gly Val Thr Thr Arg
485 490 495

acc ttc tgc gga act ccg gac tac att gcc cca gag ata atc gct tac 1536
Thr Phe Cys Gly Thr Pro Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr
500 505 510

cag ccg tac ggg aag tct gta gat tgg tgg gcg tac ggt gtg ctg ctg 1584

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Gln Pro Tyr Gly Lys Ser Val Asp Trp Trp Ala Tyr Gly Val Leu Leu
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tac gag atg cta gcc ggg cag cct ccg ttt gat ggt gaa gat gaa gat     1632
Tyr Glu Met Leu Ala Gly Gln Pro Pro Phe Asp Gly Glu Asp Glu Asp
    530                 535                 540

gaa ctg ttt cag tct ata atg gag cac aac gtg tcc tac ccc aaa tcc     1680
Glu Leu Phe Gln Ser Ile Met Glu His Asn Val Ser Tyr Pro Lys Ser
545                 550                 555                 560

ttg tcc aag gaa gcc gtc tcc atc tgc aaa gga ctt atg acc aaa cag     1728
Leu Ser Lys Glu Ala Val Ser Ile Cys Lys Gly Leu Met Thr Lys Gln
                565                 570                 575

cct gcc aag cga ctg ggc tgc ggg ccc gag gga gag agg gat gtc aga     1776
Pro Ala Lys Arg Leu Gly Cys Gly Pro Glu Gly Glu Arg Asp Val Arg
            580                 585                 590

gag cat gcc ttc ttc agg agg atc gac tgg gag aaa ctg gag aac agg     1824
Glu His Ala Phe Phe Arg Arg Ile Asp Trp Glu Lys Leu Glu Asn Arg
        595                 600                 605

gag atc caa cca cca ttc aag ccc aaa gtg tgt ggc aaa gga gca gaa     1872
Glu Ile Gln Pro Pro Phe Lys Pro Lys Val Cys Gly Lys Gly Ala Glu
    610                 615                 620

aac ttt gac aag ttc ttc acg cga gga cag cct gtc tta aca cca cca     1920
Asn Phe Asp Lys Phe Phe Thr Arg Gly Gln Pro Val Leu Thr Pro Pro
625                 630                 635                 640

gat cag ctg gtc att gct aac ata gac caa tct gat ttt gaa ggg ttc     1968
Asp Gln Leu Val Ile Ala Asn Ile Asp Gln Ser Asp Phe Glu Gly Phe
                645                 650                 655

tcg tat gtc aac ccc cag ttt gtg cac cca atc ttg caa agt gca gta     2016
Ser Tyr Val Asn Pro Gln Phe Val His Pro Ile Leu Gln Ser Ala Val
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ggg cgc gcc atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca     2064
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Gly Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
675 680 685

att ctt gtt gaa tta gat ggc gat gtt aat ggg caa aaa ttc tct gtt 2112
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val
690 695 700

agt gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa 2160
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
705 710 715 720

ttt att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc 2208
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
725 730 735

act act ctc act tat ggt gtt caa tgc ttt tct aga tac cca gat cat 2256
Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
740 745 750

atg aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta 2304
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
755 760 765

cag gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt 2352
Gln Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg
770 775 780

gct gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta 2400
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
785 790 795 800

aaa ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg 2448
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met
805 810 815

gaa tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca 2496
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro
820 825 830

aag aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat 2544

Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp
835 840 845

gga agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc 2592
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
850 855 860

gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct 2640
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
865 870 875 880

gcc ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt 2688
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu
885 890 895

gag ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac 2736
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20 25 30
Glu Val Lys Asp His Lys Phe Ile Ala Arg Phe Phe Lys Gln Pro Thr
35 40 45
Phe Cys Ser His Cys Thr Asp Phe Ile Trp Gly Phe Gly Lys Gln Gly
50 55 60
Phe Gln Cys Gln Val Cys Cys Phe Val Val His Lys Arg Cys His Glu
65 70 75 80

Phe Val Thr Phe Ser Cys Pro Gly Ala Asp Lys Gly Pro Asp Thr Asp
85 90 95

Asp Pro Arg Ser Lys His Lys Phe Lys Ile His Thr Tyr Gly Ser Pro
100 105 110

Thr Phe Cys Asp His Cys Gly Ser Leu Leu Tyr Gly Leu Ile His Gln
115 120 125

Gly Met Lys Cys Asp Thr Cys Asp Met Asn Val His Asn Gln Cys Val
130 135 140

Ile Asn Asp Pro Ser Leu Cys Gly Met Asp His Thr Glu Lys Arg Gly
145 150 155 160

Arg Ile Tyr Leu Lys Ala Glu Val Thr Asp Glu Lys Leu His Val Thr
165 170 175

Val Arg Asp Ala Lys Asn Leu Ile Pro Met Asp Pro Asn Gly Leu Ser
180 185 190

Asp Pro Tyr Val Lys Leu Lys Leu Ile Pro Asp Pro Lys Asn Glu Ser
195 200 205

Lys Gln Lys Thr Lys Thr Ile Arg Ser Asn Leu Asn Pro Gln Trp Asn
210 215 220

Glu Ser Phe Thr Phe Lys Leu Lys Pro Ser Asp Lys Asp Arg Arg Leu
225 230 235 240

Ser Val Glu Ile Trp Asp Trp Asp Arg Thr Thr Arg Asn Asp Phe Met
245 250 255

Gly Ser Leu Ser Phe Gly Val Ser Glu Leu Met Lys Met Pro Ala Ser
260 265 270

Gly Trp Tyr Lys Ala His Asn Gln Glu Glu Gly Glu Tyr Tyr Asn Val
275 280 285

Pro Ile Pro Glu Gly Asp Glu Glu Gly Asn Met Glu Leu Arg Gln Lys
290 295 300

Phe Glu Lys Ala Lys Leu Gly Pro Val Gly Asn Lys Val Ile Ser Pro
305 310 315 320

Ser Glu Asp Arg Lys Gln Pro Ser Asn Asn Leu Asp Arg Val Lys Leu
325 330 335

Thr Asp Phe Asn Phe Leu Met Val Leu Gly Lys Gly Ser Phe Gly Lys
340 345 350

Val Met Leu Ala Asp Arg Lys Gly Thr Glu Glu Leu Tyr Ala Ile Lys
355 360 365

Ile Leu Lys Lys Asp Val Val Ile Gln Asp Asp Asp Val Glu Cys Thr
370 375 380

Met Val Glu Lys Arg Val Leu Ala Leu Leu Asp Lys Pro Pro Phe Leu
385 390 395 400

Thr Gln Leu His Ser Cys Phe Gln Thr Val Asp Arg Leu Tyr Phe Val
405 410 415

Met Glu Tyr Val Asn Gly Gly Asp Leu Met Tyr His Ile Gln Gln Val
420 425 430

Gly Lys Phe Lys Glu Pro Gln Ala Val Phe Tyr Ala Ala Glu Ile Ser
435 440 445

Ile Gly Leu Phe Phe Leu His Lys Arg Gly Ile Ile Tyr Arg Asp Leu
450 455 460

Lys Leu Asn Asn Val Met Leu Asn Ser Glu Gly His Ile Lys Ile Ala
465 470 475 480

Asp Phe Gly Met Cys Lys Glu His Met Met Asp Gly Val Thr Thr Arg
485 490 495

Thr Phe Cys Gly Thr Pro Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr
500 505 510

Gln Pro Tyr Gly Lys Ser Val Asp Trp Trp Ala Tyr Gly Val Leu Leu
515 520 525

Tyr Glu Met Leu Ala Gly Gln Pro Pro Phe Asp Gly Glu Asp Glu Asp
530 535 540

Glu Leu Phe Gln Ser Ile Met Glu His Asn Val Ser Tyr Pro Lys Ser
545 550 555 560

Leu Ser Lys Glu Ala Val Ser Ile Cys Lys Gly Leu Met Thr Lys Gln
565 570 575

Pro Ala Lys Arg Leu Gly Cys Gly Pro Glu Gly Glu Arg Asp Val Arg
580 585 590

Glu His Ala Phe Phe Arg Arg Ile Asp Trp Glu Lys Leu Glu Asn Arg
595 600 605

Glu Ile Gln Pro Pro Phe Lys Pro Lys Val Cys Gly Lys Gly Ala Glu
610 615 620

Asn Phe Asp Lys Phe Phe Thr Arg Gly Gln Pro Val Leu Thr Pro Pro
625 630 635 640

Asp Gln Leu Val Ile Ala Asn Ile Asp Gln Ser Asp Phe Glu Gly Phe
645 650 655

Ser Tyr Val Asn Pro Gln Phe Val His Pro Ile Leu Gln Ser Ala Val
660 665 670

Gly Arg Ala Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
675 680 685

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val
690 695 700

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
705 710 715 720

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Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
                725                 730                 735
Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
            740                 745                 750
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
        755                 760                 765
Gln Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg
    770                 775                 780
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
785                 790                 795                 800
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met
                805                 810                 815
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro
            820                 825                 830
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp
        835                 840                 845
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    850                 855                 860
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
865                 870                 875                 880
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu
                885                 890                 895
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr
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Lys Pro Gln Glu
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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

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gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc      576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg      624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc      672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc      720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga gct caa gct tcg aat tca acc atg gcg gcg gcg      768
Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ala Ala Ala
                245                 250                 255

gcg gct cag ggg ggc ggg ggc ggg gag ccc cgt aga acc gag ggg gtc      816
Ala Ala Gln Gly Gly Gly Gly Gly Glu Pro Arg Arg Thr Glu Gly Val
            260                 265                 270

ggc ccg ggg gtc ccg ggg gag gtg gag atg gtg aag ggg cag ccg ttc      864
Gly Pro Gly Val Pro Gly Glu Val Glu Met Val Lys Gly Gln Pro Phe
        275                 280                 285

gac gtg ggc ccg cgc tac acg cag ttg cag tac atc ggc gag ggc gcg      912
Asp Val Gly Pro Arg Tyr Thr Gln Leu Gln Tyr Ile Gly Glu Gly Ala
    290                 295                 300

tac ggc atg gtc agc tcg gcc tat gac cac gtg cgc aag act cgc gtg      960
Tyr Gly Met Val Ser Ser Ala Tyr Asp His Val Arg Lys Thr Arg Val
305                 310                 315                 320

gcc atc aag aag atc agc ccc ttc gaa cat cag acc tac tgc cag cgc     1008
Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr Cys Gln Arg
                325                 330                 335
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acg ctc cgg gag atc cag atc ctg ctg cgc ttc cgc cat gag aat gtc 1056
Thr Leu Arg Glu Ile Gln Ile Leu Leu Arg Phe Arg His Glu Asn Val
340 345 350

atc ggc atc cga gac att ctg cgg gcg tcc acc ctg gaa gcc atg aga 1104
Ile Gly Ile Arg Asp Ile Leu Arg Ala Ser Thr Leu Glu Ala Met Arg
355 360 365

gat gtc tac att gtg cag gac ctg atg gag act gac ctg tac aag ttg 1152
Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu Tyr Lys Leu
370 375 380

ctg aaa agc cag cag ctg agc aat gac cat atc tgc tac ttc ctc tac 1200
Leu Lys Ser Gln Gln Leu Ser Asn Asp His Ile Cys Tyr Phe Leu Tyr
385 390 395 400

cag atc ctg cgg ggc ctc aag tac atc cac tcc gcc aac gtg ctc cac 1248
Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asn Val Leu His
405 410 415

cga gat cta aag ccc tcc aac ctg ctc agc aac acc acc tgc gac ctt 1296
Arg Asp Leu Lys Pro Ser Asn Leu Leu Ser Asn Thr Thr Cys Asp Leu
420 425 430

aag att tgt gat ttc ggc ctg gcc cgg att gcc gat cct gag cat gac 1344
Lys Ile Cys Asp Phe Gly Leu Ala Arg Ile Ala Asp Pro Glu His Asp
435 440 445

cac acc ggc ttc ctg acg gag tat gtg gct acg cgc tgg tac cgg gcc 1392
His Thr Gly Phe Leu Thr Glu Tyr Val Ala Thr Arg Trp Tyr Arg Ala
450 455 460

cca gag atc atg ctg aac tcc aag ggc tat acc aag tcc atc gac atc 1440
Pro Glu Ile Met Leu Asn Ser Lys Gly Tyr Thr Lys Ser Ile Asp Ile
465 470 475 480

tgg tct gtg ggc tgc att ctg gct gag atg ctc tct aac cgg ccc atc 1488
Trp Ser Val Gly Cys Ile Leu Ala Glu Met Leu Ser Asn Arg Pro Ile
485 490 495

ttc cct ggc aag cac tac ctg gat cag ctc aac cac att ctg ggc atc 1536
Phe Pro Gly Lys His Tyr Leu Asp Gln Leu Asn His Ile Leu Gly Ile
500 505 510

ctg ggc tcc cca tcc cag gag gac ctg aat tgt atc atc aac atg aag 1584
Leu Gly Ser Pro Ser Gln Glu Asp Leu Asn Cys Ile Ile Asn Met Lys
515 520 525

gcc cga aac tac cta cag tct ctg ccc tcc aag acc aag gtg gct tgg 1632
Ala Arg Asn Tyr Leu Gln Ser Leu Pro Ser Lys Thr Lys Val Ala Trp
530 535 540

gcc aag ctt ttc ccc aag tca gac tcc aaa gcc ctt gac ctg ctg gac 1680
Ala Lys Leu Phe Pro Lys Ser Asp Ser Lys Ala Leu Asp Leu Leu Asp
545 550 555 560

cgg atg tta acc ttt aac ccc aat aaa cgg atc aca gtg gag gaa gcg 1728
Arg Met Leu Thr Phe Asn Pro Asn Lys Arg Ile Thr Val Glu Glu Ala
565 570 575

ctg gct cac ccc tac ctg gag cag tac tat gac ccg acg gat gag cca 1776
Leu Ala His Pro Tyr Leu Glu Gln Tyr Tyr Asp Pro Thr Asp Glu Pro
580 585 590

gtg gcc gag gag ccc ttc acc ttc gcc atg gag ctg gat gac cta cct 1824
Val Ala Glu Glu Pro Phe Thr Phe Ala Met Glu Leu Asp Asp Leu Pro
595 600 605

aag gag cgg ctg aag gag ctc atc ttc cag gag aca gca cgc ttc cag 1872
Lys Glu Arg Leu Lys Glu Leu Ile Phe Gln Glu Thr Ala Arg Phe Gln
610 615 620

ccc gga gtg ctg gag gcc ccc tag 1896
Pro Gly Val Leu Glu Ala Pro *
625 630

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<211> 631

<212> PRT

<213> Aequorea victoria and human

<400> 6

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                  10                  15
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240
Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ala Ala Ala
                245                 250                 255
Ala Ala Gln Gly Gly Gly Gly Gly Glu Pro Arg Arg Thr Glu Gly Val
            260                 265                 270
Gly Pro Gly Val Pro Gly Glu Val Glu Met Val Lys Gly Gln Pro Phe
        275                 280                 285
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Asp Val Gly Pro Arg Tyr Thr Gln Leu Gln Tyr Ile Gly Glu Gly Ala
    290                 295                 300
Tyr Gly Met Val Ser Ser Ala Tyr Asp His Val Arg Lys Thr Arg Val
305                 310                 315                 320
Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr Cys Gln Arg
                325                 330                 335
Thr Leu Arg Glu Ile Gln Ile Leu Leu Arg Phe Arg His Glu Asn Val
            340                 345                 350
Ile Gly Ile Arg Asp Ile Leu Arg Ala Ser Thr Leu Glu Ala Met Arg
        355                 360                 365
Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu Tyr Lys Leu
    370                 375                 380
Leu Lys Ser Gln Gln Leu Ser Asn Asp His Ile Cys Tyr Phe Leu Tyr
385                 390                 395                 400
Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asn Val Leu His
                405                 410                 415
Arg Asp Leu Lys Pro Ser Asn Leu Leu Ser Asn Thr Thr Cys Asp Leu
            420                 425                 430
Lys Ile Cys Asp Phe Gly Leu Ala Arg Ile Ala Asp Pro Glu His Asp
        435                 440                 445
His Thr Gly Phe Leu Thr Glu Tyr Val Ala Thr Arg Trp Tyr Arg Ala
    450                 455                 460
Pro Glu Ile Met Leu Asn Ser Lys Gly Tyr Thr Lys Ser Ile Asp Ile
465                 470                 475                 480
Trp Ser Val Gly Cys Ile Leu Ala Glu Met Leu Ser Asn Arg Pro Ile
                485                 490                 495
Phe Pro Gly Lys His Tyr Leu Asp Gln Leu Asn His Ile Leu Gly Ile
            500                 505                 510
Leu Gly Ser Pro Ser Gln Glu Asp Leu Asn Cys Ile Ile Asn Met Lys
        515                 520                 525
Ala Arg Asn Tyr Leu Gln Ser Leu Pro Ser Lys Thr Lys Val Ala Trp
    530                 535                 540
Ala Lys Leu Phe Pro Lys Ser Asp Ser Lys Ala Leu Asp Leu Leu Asp
545                 550                 555                 560
Arg Met Leu Thr Phe Asn Pro Asn Lys Arg Ile Thr Val Glu Glu Ala
                565                 570                 575
Leu Ala His Pro Tyr Leu Glu Gln Tyr Tyr Asp Pro Thr Asp Glu Pro
            580                 585                 590
Val Ala Glu Glu Pro Phe Thr Phe Ala Met Glu Leu Asp Asp Leu Pro
        595                 600                 605
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Lys Glu Arg Leu Lys Glu Leu Ile Phe Gln Glu Thr Ala Arg Phe Gln
610 615 620

Pro Gly Val Leu Glu Ala Pro
625 630

<210> 7
<211> 2160
<212> DNA
<213> Aequorea victoria and human

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<400> 7

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly

115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn

145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser

165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly

180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu

195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe

210 215 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc 720

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser

225 230 235 240

gga ctc aga tct cga gct caa gct tcg aat tcg acc atg tcg tcc atc 768

Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ser Ser Ile

245 250 255

ttg cca ttc acg ccg cca gtt gtg aag aga ctg ctg gga tgg aag aag 816

Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu Gly Trp Lys Lys
260 265 270

tca gct ggt ggg tct gga gga gca ggc gga gga gag cag aat ggg cag 864
Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu Gln Asn Gly Gln
275 280 285

gaa gaa aag tgg tgt gag aaa gca gtg aaa agt ctg gtg aag aag cta 912
Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu Val Lys Lys Leu
290 295 300

aag aaa aca gga cga tta gat gag ctt gag aaa gcc atc acc act caa 960
Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala Ile Thr Thr Gln
305 310 315 320

aac tgt aat act aaa tgt gtt acc ata cca agc act tgc tct gaa att 1008
Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr Cys Ser Glu Ile
325 330 335

tgg gga ctg agt aca cca aat acg ata gat cag tgg gat aca aca ggc 1056
Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp Asp Thr Thr Gly
340 345 350

ctt tac agc ttc tct gaa caa acc agg tct ctt gat ggt cgt ctc cag 1104
Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp Gly Arg Leu Gln
355 360 365

gta tcc cat cga aaa gga ttg cca cat gtt ata tat tgc cga tta tgg 1152
Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr Cys Arg Leu Trp
370 375 380

cgc tgg cct gat ctt cac agt cat cat gaa ctc aag gca att gaa aac 1200
Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys Ala Ile Glu Asn
385 390 395 400

tgc gaa tat gct ttt aat ctt aaa aag gat gaa gta tgt gta aac cct 1248
Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val Cys Val Asn Pro
405 410 415

tac cac tat cag aga gtt gag aca cca gtt ttg cct cca gta tta gtg 1296

Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro Pro Val Leu Val
420 425 430

ccc cga cac acc gag atc cta aca gaa ctt ccg cct ctg gat gac tat 1344
Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro Leu Asp Asp Tyr
435 440 445

act cac tcc att cca gaa aac act aac ttc cca gca gga att gag cca 1392
Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala Gly Ile Glu Pro
450 455 460

cag agt aat tat att cca gaa acg cca cct cct gga tat atc agt gaa 1440
Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly Tyr Ile Ser Glu
465 470 475 480

gat gga gaa aca agt gac caa cag ttg aat caa agt atg gac aca ggc 1488
Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser Met Asp Thr Gly
485 490 495

tct cca gca gaa cta tct cct act act ctt tcc cct gtt aat cat agc 1536
Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro Val Asn His Ser
500 505 510

ttg gat tta cag cca gtt act tac tca gaa cct gca ttt tgg tgt tca 1584
Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala Phe Trp Cys Ser
515 520 525

ata gca tat tat gaa tta aat cag agg gtt gga gaa acc ttc cat gca 1632
Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu Thr Phe His Ala
530 535 540

tca cag ccc tca ctc act gta gat ggc ttt aca gac cca tca aat tca 1680
Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp Pro Ser Asn Ser
545 550 555 560

gag agg ttc tgc tta ggt tta ctc tcc aat gtt aac cga aat gcc acg 1728
Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn Arg Asn Ala Thr
565 570 575

gta gaa atg aca aga agg cat ata gga aga gga gtg cgc tta tac tac 1776

Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val Arg Leu Tyr Tyr
580 585 590

ata ggt ggg gaa gtt ttt gct gag tgc cta agt gat agt gca atc ttt 1824
Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp Ser Ala Ile Phe
595 600 605

gtg cag agc ccc aat tgt aat cag aga tat ggc tgg cac cct gca aca 1872
Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp His Pro Ala Thr
610 615 620

gtg tgt aaa att cca cca ggc tgt aat ctg aag atc ttc aac aac cag 1920
Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile Phe Asn Asn Gln
625 630 635 640

gaa ttt gct gct ctt ctg gct cag tct gtt aat cag ggt ttt gaa gcc 1968
Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln Gly Phe Glu Ala
645 650 655

gtc tat cag cta act aga atg tgc acc ata aga atg agt ttt gtg aaa 2016
Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met Ser Phe Val Lys
660 665 670

ggg tgg gga gca gaa tac cga agg cag acg gta aca agt act cct tgc 2064
Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr Ser Thr Pro Cys
675 680 685

tgg att gaa ctt cat ctg aat gga cct cta cag tgg ttg gac aaa gta 2112
Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp Leu Asp Lys Val
690 695 700

tta act cag atg gga tcc cct tca gtg cgt tgc tca agc atg tca taa 2160
Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser Ser Met Ser *
705 710 715

<210> 8
<211> 719
<212> PRT
<213> Aequorea victoria and human

<400> 8

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Gln Ala Ser Asn Ser Thr Met Ser Ser Ile
245 250 255

Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu Gly Trp Lys Lys
260 265 270

Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu Gln Asn Gly Gln
275 280 285

Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu Val Lys Lys Leu
290 295 300

Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala Ile Thr Thr Gln
305 310 315 320

Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr Cys Ser Glu Ile
325 330 335

Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp Asp Thr Thr Gly
340 345 350

Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp Gly Arg Leu Gln
355 360 365

Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr Cys Arg Leu Trp
370 375 380

Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys Ala Ile Glu Asn
385 390 395 400

Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val Cys Val Asn Pro
405 410 415

Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro Pro Val Leu Val
420 425 430

Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro Leu Asp Asp Tyr
435 440 445

Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala Gly Ile Glu Pro
450 455 460

Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly Tyr Ile Ser Glu
465 470 475 480

Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser Met Asp Thr Gly
485 490 495

Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro Val Asn His Ser
500 505 510

Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala Phe Trp Cys Ser
515 520 525

Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu Thr Phe His Ala
530 535 540

Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp Pro Ser Asn Ser
545 550 555 560

Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn Arg Asn Ala Thr
565 570 575

Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val Arg Leu Tyr Tyr
580 585 590

Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp Ser Ala Ile Phe
595 600 605

Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp His Pro Ala Thr
610 615 620

Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile Phe Asn Asn Gln
625 630 635 640
Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln Gly Phe Glu Ala
645 650 655
Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met Ser Phe Val Lys
660 665 670
Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr Ser Thr Pro Cys
675 680 685
Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp Leu Asp Lys Val
690 695 700
Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser Ser Met Ser
705 710 715

<210> 9
<211> 2157
<212> DNA
<213> Aequorea victoria and human

<220>
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<222> (1)...(2157)

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Met Ser Ser Ile Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu
1 5 10 15

gga tgg aag aag tca gct ggt ggg tct gga gga gca ggc gga gga gag 96
Gly Trp Lys Lys Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu
20 25 30

cag aat ggg cag gaa gaa aag tgg tgt gag aaa gca gtg aaa agt ctg 144
Gln Asn Gly Gln Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu
35 40 45

gtg aag aag cta aag aaa aca gga cga tta gat gag ctt gag aaa gcc 192
Val Lys Lys Leu Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala
50 55 60

atc acc act caa aac tgt aat act aaa tgt gtt acc ata cca agc act 240

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Ile Thr Thr Gln Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr
65                  70                  75                  80

tgc tct gaa att tgg gga ctg agt aca cca aat acg ata gat cag tgg      288
Cys Ser Glu Ile Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp
                85                  90                  95

gat aca aca ggc ctt tac agc ttc tct gaa caa acc agg tct ctt gat      336
Asp Thr Thr Gly Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp
            100                 105                 110

ggt cgt ctc cag gta tcc cat cga aaa gga ttg cca cat gtt ata tat      384
Gly Arg Leu Gln Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr
        115                 120                 125

tgc cga tta tgg cgc tgg cct gat ctt cac agt cat cat gaa ctc aag      432
Cys Arg Leu Trp Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys
    130                 135                 140

gca att gaa aac tgc gaa tat gct ttt aat ctt aaa aag gat gaa gta      480
Ala Ile Glu Asn Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val
145                 150                 155                 160

tgt gta aac cct tac cac tat cag aga gtt gag aca cca gtt ttg cct      528
Cys Val Asn Pro Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro
                165                 170                 175

cca gta tta gtg ccc cga cac acc gag atc cta aca gaa ctt ccg cct      576
Pro Val Leu Val Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro
            180                 185                 190

ctg gat gac tat act cac tcc att cca gaa aac act aac ttc cca gca      624
Leu Asp Asp Tyr Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala
        195                 200                 205

gga att gag cca cag agt aat tat att cca gaa acg cca cct cct gga      672
Gly Ile Glu Pro Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly
    210                 215                 220

tat atc agt gaa gat gga gaa aca agt gac caa cag ttg aat caa agt      720
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Tyr Ile Ser Glu Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser
225 230 235 240

atg gac aca ggc tct cca gca gaa cta tct cct act act ctt tcc cct 768
Met Asp Thr Gly Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro
245 250 255

gtt aat cat agc ttg gat tta cag cca gtt act tac tca gaa cct gca 816
Val Asn His Ser Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala
260 265 270

ttt tgg tgt tca ata gca tat tat gaa tta aat cag agg gtt gga gaa 864
Phe Trp Cys Ser Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu
275 280 285

acc ttc cat gca tca cag ccc tca ctc act gta gat ggc ttt aca gac 912
Thr Phe His Ala Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp
290 295 300

cca tca aat tca gag agg ttc tgc tta ggt tta ctc tcc aat gtt aac 960
Pro Ser Asn Ser Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn
305 310 315 320

cga aat gcc acg gta gaa atg aca aga agg cat ata gga aga gga gtg 1008
Arg Asn Ala Thr Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val
325 330 335

cgc tta tac tac ata ggt ggg gaa gtt ttt gct gag tgc cta agt gat 1056
Arg Leu Tyr Tyr Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp
340 345 350

agt gca atc ttt gtg cag agc ccc aat tgt aat cag aga tat ggc tgg 1104
Ser Ala Ile Phe Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp
355 360 365

cac cct gca aca gtg tgt aaa att cca cca ggc tgt aat ctg aag atc 1152
His Pro Ala Thr Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile
370 375 380

ttc aac aac cag gaa ttt gct gct ctt ctg gct cag tct gtt aat cag 1200

Phe Asn Asn Gln Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln
385 390 395 400

ggt ttt gaa gcc gtc tat cag cta act aga atg tgc acc ata aga atg 1248
Gly Phe Glu Ala Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met
405 410 415

agt ttt gtg aaa ggg tgg gga gca gaa tac cga agg cag acg gta aca 1296
Ser Phe Val Lys Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr
420 425 430

agt act cct tgc tgg att gaa ctt cat ctg aat gga cct cta cag tgg 1344
Ser Thr Pro Cys Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp
435 440 445

ttg gac aaa gta tta act cag atg gga tcc cct tca gtg cgt tgc tca 1392
Leu Asp Lys Val Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser
450 455 460

agc atg tca tgg gta ccg cgg gcc cgg gat cca ccg gtc gcc acc atg 1440
Ser Met Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met
465 470 475 480

gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc 1488
Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
485 490 495

gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag 1536
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
500 505 510

ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc 1584
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
515 520 525

acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg 1632
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
530 535 540

acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag cag 1680

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
545 550 555 560

cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc 1728
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
565 570 575

acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg 1776
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
580 585 590

aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc 1824
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
595 600 605

gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac 1872
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
610 615 620

tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac ggc 1920
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
625 630 635 640

atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg 1968
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
645 650 655

cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc 2016
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
660 665 670

gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc 2064
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
675 680 685

aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg 2112
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
690 695 700

acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa 2157

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys *
705 710 715

<210> 10
<211> 718
<212> PRT
<213> Aequorea victoria and human

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Met Ser Ser Ile Leu Pro Phe Thr Pro Pro Val Val Lys Arg Leu Leu
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Gly Trp Lys Lys Ser Ala Gly Gly Ser Gly Gly Ala Gly Gly Gly Glu
20 25 30
Gln Asn Gly Gln Glu Glu Lys Trp Cys Glu Lys Ala Val Lys Ser Leu
35 40 45
Val Lys Lys Leu Lys Lys Thr Gly Arg Leu Asp Glu Leu Glu Lys Ala
50 55 60
Ile Thr Thr Gln Asn Cys Asn Thr Lys Cys Val Thr Ile Pro Ser Thr
65 70 75 80
Cys Ser Glu Ile Trp Gly Leu Ser Thr Pro Asn Thr Ile Asp Gln Trp
85 90 95
Asp Thr Thr Gly Leu Tyr Ser Phe Ser Glu Gln Thr Arg Ser Leu Asp
100 105 110
Gly Arg Leu Gln Val Ser His Arg Lys Gly Leu Pro His Val Ile Tyr
115 120 125
Cys Arg Leu Trp Arg Trp Pro Asp Leu His Ser His His Glu Leu Lys
130 135 140
Ala Ile Glu Asn Cys Glu Tyr Ala Phe Asn Leu Lys Lys Asp Glu Val
145 150 155 160
Cys Val Asn Pro Tyr His Tyr Gln Arg Val Glu Thr Pro Val Leu Pro
165 170 175
Pro Val Leu Val Pro Arg His Thr Glu Ile Leu Thr Glu Leu Pro Pro
180 185 190
Leu Asp Asp Tyr Thr His Ser Ile Pro Glu Asn Thr Asn Phe Pro Ala
195 200 205
Gly Ile Glu Pro Gln Ser Asn Tyr Ile Pro Glu Thr Pro Pro Pro Gly
210 215 220
Tyr Ile Ser Glu Asp Gly Glu Thr Ser Asp Gln Gln Leu Asn Gln Ser
225 230 235 240

Met Asp Thr Gly Ser Pro Ala Glu Leu Ser Pro Thr Thr Leu Ser Pro
245 250 255

Val Asn His Ser Leu Asp Leu Gln Pro Val Thr Tyr Ser Glu Pro Ala
260 265 270

Phe Trp Cys Ser Ile Ala Tyr Tyr Glu Leu Asn Gln Arg Val Gly Glu
275 280 285

Thr Phe His Ala Ser Gln Pro Ser Leu Thr Val Asp Gly Phe Thr Asp
290 295 300

Pro Ser Asn Ser Glu Arg Phe Cys Leu Gly Leu Leu Ser Asn Val Asn
305 310 315 320

Arg Asn Ala Thr Val Glu Met Thr Arg Arg His Ile Gly Arg Gly Val
325 330 335

Arg Leu Tyr Tyr Ile Gly Gly Glu Val Phe Ala Glu Cys Leu Ser Asp
340 345 350

Ser Ala Ile Phe Val Gln Ser Pro Asn Cys Asn Gln Arg Tyr Gly Trp
355 360 365

His Pro Ala Thr Val Cys Lys Ile Pro Pro Gly Cys Asn Leu Lys Ile
370 375 380

Phe Asn Asn Gln Glu Phe Ala Ala Leu Leu Ala Gln Ser Val Asn Gln
385 390 395 400

Gly Phe Glu Ala Val Tyr Gln Leu Thr Arg Met Cys Thr Ile Arg Met
405 410 415

Ser Phe Val Lys Gly Trp Gly Ala Glu Tyr Arg Arg Gln Thr Val Thr
420 425 430

Ser Thr Pro Cys Trp Ile Glu Leu His Leu Asn Gly Pro Leu Gln Trp
435 440 445

Leu Asp Lys Val Leu Thr Gln Met Gly Ser Pro Ser Val Arg Cys Ser
450 455 460

Ser Met Ser Trp Val Pro Arg Ala Arg Asp Pro Pro Val Ala Thr Met
465 470 475 480

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
485 490 495

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
500 505 510

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
515 520 525

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
530 535 540

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
545 550 555 560

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
565 570 575

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
580 585 590

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
595 600 605

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
610 615 620

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
625 630 635 640

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
645 650 655

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
660 665 670

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
675 680 685

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
690 695 700

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
705 710 715

<210> 11
<211> 1908
<212> DNA
<213> Aequorea victoria and human

<220>
<221> CDS
<222> (1)...(1908)

<400> 11

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624

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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc      672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc      720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240

gga ctc aga tct cga gct caa gct tcc atg agc gag acg gtc atc atg      768
Gly Leu Arg Ser Arg Ala Gln Ala Ser Met Ser Glu Thr Val Ile Met
                245                 250                 255

agc gag acg gtc atc tgt tcc agc cgg gcc act gtg atg ctt tat gat      816
Ser Glu Thr Val Ile Cys Ser Ser Arg Ala Thr Val Met Leu Tyr Asp
            260                 265                 270

gat ggc aac aag cga tgg ctc cct gct ggc acg ggt ccc cag gcc ttc      864
Asp Gly Asn Lys Arg Trp Leu Pro Ala Gly Thr Gly Pro Gln Ala Phe
        275                 280                 285

agc cgc gtc cag atc tac cac aac ccc acg gcc aat tcc ttt cgc gtc      912
Ser Arg Val Gln Ile Tyr His Asn Pro Thr Ala Asn Ser Phe Arg Val
    290                 295                 300

gtg ggc cgg aag atg cag ccc gac cag cag gtg gtc atc aac tgt gcc      960
Val Gly Arg Lys Met Gln Pro Asp Gln Gln Val Val Ile Asn Cys Ala
305                 310                 315                 320

atc gtc cgg ggt gtc aag tat aac cag gcc acc ccc aac ttc cat cag     1008
Ile Val Arg Gly Val Lys Tyr Asn Gln Ala Thr Pro Asn Phe His Gln
                325                 330                 335

tgg cgc gac gct cgc cag gtc tgg ggc ctc aac ttc ggc agc aag gag     1056
Trp Arg Asp Ala Arg Gln Val Trp Gly Leu Asn Phe Gly Ser Lys Glu
            340                 345                 350

gat gcg gcc cag ttt gcc gcc ggc atg gcc agt gcc cta gag gcg ttg     1104
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Asp Ala Ala Gln Phe Ala Ala Gly Met Ala Ser Ala Leu Glu Ala Leu
355 360 365

gaa gga ggt ggg ccc cct cca ccc cca gca ctt ccc acc tgg tcg gtc 1152
Glu Gly Gly Gly Pro Pro Pro Pro Pro Ala Leu Pro Thr Trp Ser Val
370 375 380

ccg aac ggc ccc tcc ccg gag gag gtg gag cag cag aaa agg cag cag 1200
Pro Asn Gly Pro Ser Pro Glu Glu Val Glu Gln Gln Lys Arg Gln Gln
385 390 395 400

ccc ggc ccg tcg gag cac ata gag cgc cgg gtc tcc aat gca gga ggc 1248
Pro Gly Pro Ser Glu His Ile Glu Arg Arg Val Ser Asn Ala Gly Gly
405 410 415

cca cct gct ccc ccc gct ggg ggt cca ccc cca cca cca gga cct ccc 1296
Pro Pro Ala Pro Pro Ala Gly Gly Pro Pro Pro Pro Pro Gly Pro Pro
420 425 430

cct cct cca ggt ccc ccc cca ccc cca ggt ttg ccc cct tcg ggg gtc 1344
Pro Pro Pro Gly Pro Pro Pro Pro Pro Gly Leu Pro Pro Ser Gly Val
435 440 445

cca gct gca gcg cac gga gca ggg gga gga cca ccc cct gca ccc cct 1392
Pro Ala Ala Ala His Gly Ala Gly Gly Gly Pro Pro Pro Ala Pro Pro
450 455 460

ctc ccg gca gca cag ggc cct ggt ggt ggg gga gct ggg gcc cca ggc 1440
Leu Pro Ala Ala Gln Gly Pro Gly Gly Gly Gly Ala Gly Ala Pro Gly
465 470 475 480

ctg gcc gca gct att gct gga gcc aaa ctc agg aaa gtc agc aag cag 1488
Leu Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Lys Gln
485 490 495

gag gag gcc tca ggg ggg ccc aca gcc ccc aaa gct gag agt ggt cga 1536
Glu Glu Ala Ser Gly Gly Pro Thr Ala Pro Lys Ala Glu Ser Gly Arg
500 505 510

agc gga ggt ggg gga ctc atg gaa gag atg aac gcc atg ctg gcc cgg 1584

Ser Gly Gly Gly Gly Leu Met Glu Glu Met Asn Ala Met Leu Ala Arg
515 520 525

aga agg aaa gcc acg caa gtt ggg gag aaa acc ccc aag gat gaa tct 1632
Arg Arg Lys Ala Thr Gln Val Gly Glu Lys Thr Pro Lys Asp Glu Ser
530 535 540

gcc aat cag gag gag cca gag gcc aga gtc ccg gcc cag agt gaa tct 1680
Ala Asn Gln Glu Glu Pro Glu Ala Arg Val Pro Ala Gln Ser Glu Ser
545 550 555 560

gtg cgg aga ccc tgg gag aag aac agc aca acc ttg cca agg atg aag 1728
Val Arg Arg Pro Trp Glu Lys Asn Ser Thr Thr Leu Pro Arg Met Lys
565 570 575

tcg tct tct tcg gtg acc act tcc gag acc caa ccc tgc acg ccc agc 1776
Ser Ser Ser Ser Val Thr Thr Ser Glu Thr Gln Pro Cys Thr Pro Ser
580 585 590

tcc agt gat tac tcg gac cta cag agg gtg aaa cag gag ctt ctg gaa 1824
Ser Ser Asp Tyr Ser Asp Leu Gln Arg Val Lys Gln Glu Leu Leu Glu
595 600 605

gag gtg aag aag gaa ttg cag aaa gtg aaa gag gaa atc att gaa gcc 1872
Glu Val Lys Lys Glu Leu Gln Lys Val Lys Glu Glu Ile Ile Glu Ala
610 615 620

ttc gtc cag gag ctg agg aag cgg ggt tct ccc tga 1908
Phe Val Gln Glu Leu Arg Lys Arg Gly Ser Pro *
625 630 635

<210> 12
<211> 635
<212> PRT
<213> Aequorea victoria and human

<400> 12
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240
Gly Leu Arg Ser Arg Ala Gln Ala Ser Met Ser Glu Thr Val Ile Met
245 250 255
Ser Glu Thr Val Ile Cys Ser Ser Arg Ala Thr Val Met Leu Tyr Asp
260 265 270
Asp Gly Asn Lys Arg Trp Leu Pro Ala Gly Thr Gly Pro Gln Ala Phe
275 280 285
Ser Arg Val Gln Ile Tyr His Asn Pro Thr Ala Asn Ser Phe Arg Val
290 295 300
Val Gly Arg Lys Met Gln Pro Asp Gln Gln Val Val Ile Asn Cys Ala
305 310 315 320
Ile Val Arg Gly Val Lys Tyr Asn Gln Ala Thr Pro Asn Phe His Gln
325 330 335

Trp Arg Asp Ala Arg Gln Val Trp Gly Leu Asn Phe Gly Ser Lys Glu
340 345 350

Asp Ala Ala Gln Phe Ala Ala Gly Met Ala Ser Ala Leu Glu Ala Leu
355 360 365

Glu Gly Gly Gly Pro Pro Pro Pro Pro Ala Leu Pro Thr Trp Ser Val
370 375 380

Pro Asn Gly Pro Ser Pro Glu Glu Val Glu Gln Gln Lys Arg Gln Gln
385 390 395 400

Pro Gly Pro Ser Glu His Ile Glu Arg Arg Val Ser Asn Ala Gly Gly
405 410 415

Pro Pro Ala Pro Pro Ala Gly Gly Pro Pro Pro Pro Pro Gly Pro Pro
420 425 430

Pro Pro Pro Gly Pro Pro Pro Pro Pro Gly Leu Pro Pro Ser Gly Val
435 440 445

Pro Ala Ala Ala His Gly Ala Gly Gly Gly Pro Pro Pro Ala Pro Pro
450 455 460

Leu Pro Ala Ala Gln Gly Pro Gly Gly Gly Gly Ala Gly Ala Pro Gly
465 470 475 480

Leu Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Lys Gln
485 490 495

Glu Glu Ala Ser Gly Gly Pro Thr Ala Pro Lys Ala Glu Ser Gly Arg
500 505 510

Ser Gly Gly Gly Gly Leu Met Glu Glu Met Asn Ala Met Leu Ala Arg
515 520 525

Arg Arg Lys Ala Thr Gln Val Gly Glu Lys Thr Pro Lys Asp Glu Ser
530 535 540

Ala Asn Gln Glu Glu Pro Glu Ala Arg Val Pro Ala Gln Ser Glu Ser
545 550 555 560

Val Arg Arg Pro Trp Glu Lys Asn Ser Thr Thr Leu Pro Arg Met Lys
565 570 575

Ser Ser Ser Ser Val Thr Thr Ser Glu Thr Gln Pro Cys Thr Pro Ser
580 585 590

Ser Ser Asp Tyr Ser Asp Leu Gln Arg Val Lys Gln Glu Leu Leu Glu
595 600 605

Glu Val Lys Lys Glu Leu Gln Lys Val Lys Glu Glu Ile Ile Glu Ala
610 615 620

Phe Val Gln Glu Leu Arg Lys Arg Gly Ser Pro
625 630 635

<210> 13

<211> 2394
<212> DNA
<213> Aequorea victoria and human

<220>
<221> CDS
<222> (1)...(2394)

<400> 13

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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                  10                  15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc      96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc     144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc     192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag     240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag     288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag     336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc     384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125
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atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc 720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

gga ctc aga tct cga gcc atg gac gaa ctg ttc ccc ctc atc ttc ccg 768
Gly Leu Arg Ser Arg Ala Met Asp Glu Leu Phe Pro Leu Ile Phe Pro
245 250 255

gca gag cca gcc cag gcc tct ggc ccc tat gtg gag atc att gag cag 816
Ala Glu Pro Ala Gln Ala Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln
260 265 270

ccc aag cag cgg ggc atg cgc ttc cgc tac aag tgc gag ggg cgc tcc 864
Pro Lys Gln Arg Gly Met Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser
275 280 285

gcg ggc agc atc cca ggc gag agg agc aca gat acc acc aag acc cac 912
Ala Gly Ser Ile Pro Gly Glu Arg Ser Thr Asp Thr Thr Lys Thr His
290 295 300

ccc acc atc aag atc aat ggc tac aca gga cca ggg aca gtg cgc atc 960
Pro Thr Ile Lys Ile Asn Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile
305 310 315 320

tcc ctg gtc acc aag gac cct cct cac cgg cct cac ccc cac gag ctt 1008
Ser Leu Val Thr Lys Asp Pro Pro His Arg Pro His Pro His Glu Leu
325 330 335

gta gga aag gac tgc cgg gat ggc ttc tat gag gct gag ctc tgc ccg 1056
Val Gly Lys Asp Cys Arg Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro
340 345 350

gac cgc tgc atc cac agt ttc cag aac ctg gga atc cag tgt gtg aag 1104
Asp Arg Cys Ile His Ser Phe Gln Asn Leu Gly Ile Gln Cys Val Lys
355 360 365

aag cgg gac ctg gag cag gct atc agt cag cgc atc cag acc aac aac 1152
Lys Arg Asp Leu Glu Gln Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn
370 375 380

aac ccc ttc caa gtt cct ata gaa gag cag cgt ggg gac tac gac ctg 1200
Asn Pro Phe Gln Val Pro Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu
385 390 395 400

aat gct gtg cgg ctc tgc ttc cag gtg aca gtg cgg gac cca tca ggc 1248
Asn Ala Val Arg Leu Cys Phe Gln Val Thr Val Arg Asp Pro Ser Gly
405 410 415

agg ccc ctc cgc ctg ccg cct gtc ctt cct cat ccc atc ttt gac aat 1296
Arg Pro Leu Arg Leu Pro Pro Val Leu Pro His Pro Ile Phe Asp Asn
420 425 430

cgt gcc ccc aac act gcc gag ctc aag atc tgc cga gtg aac cga aac 1344
Arg Ala Pro Asn Thr Ala Glu Leu Lys Ile Cys Arg Val Asn Arg Asn
435 440 445

tct ggc agc tgc ctc ggt ggg gat gag atc ttc cta ctg tgt gac aag 1392
Ser Gly Ser Cys Leu Gly Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys
450 455 460

gtg cag aaa gag gac att gag gtg tat ttc acg gga cca ggc tgg gag 1440
Val Gln Lys Glu Asp Ile Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu
465 470 475 480

gcc cga ggc tcc ttt tcg caa gct gat gtg cac cga caa gtg gcc att 1488
Ala Arg Gly Ser Phe Ser Gln Ala Asp Val His Arg Gln Val Ala Ile
485 490 495

gtg ttc cgg acc cct ccc tac gca gac ccc agc ctg cag gct cct gtg 1536
Val Phe Arg Thr Pro Pro Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val
500 505 510

cgt gtc tcc atg cag ctg cgg cgg cct tcc gac cgg gag ctc agt gag 1584
Arg Val Ser Met Gln Leu Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu
515 520 525

ccc atg gaa ttc cag tac ctg cca gat aca gac gat cgt cac cgg att 1632
Pro Met Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg Ile
530 535 540

gag gag aaa cgt aaa agg aca tat gag acc ttc aag agc atc atg aag 1680
Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys
545 550 555 560

aag agt cct ttc agc gga ccc acc gac ccc cgg cct cca cct cga cgc 1728
Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg
565 570 575

att gct gtg cct tcc cgc agc tca gct tct gtc ccc aag cca gca ccc 1776
Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala Pro
580 585 590

cag ccc tat ccc ttt acg tca tcc ctg agc acc atc aac tat gat gag 1824
Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu
595 600 605

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ttt ccc acc atg gtg ttt cct tct ggg cag atc agc cag gcc tcg gcc      1872
Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala
    610                 615                 620

ttg gcc ccg gcc cct ccc caa gtc ctg ccc cag gct cca gcc cct gcc      1920
Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala
625                 630                 635                 640

cct gct cca gcc atg gta tca gct ctg gcc cag gcc cca gcc cct gtc      1968
Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val
                645                 650                 655

cca gtc cta gcc cca ggc cct cct cag gct gtg gcc cca cct gcc ccc      2016
Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro
            660                 665                 670

aag ccc acc cag gct ggg gaa gga acg ctg tca gag gcc ctg ctg cag      2064
Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln
        675                 680                 685

ctg cag ttt gat gat gaa gac ctg ggg gcc ttg ctt ggc aac agc aca      2112
Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr
    690                 695                 700

gac cca gct gtg ttc aca gac ctg gca tcc gtc gac aac tcc gag ttt      2160
Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe
705                 710                 715                 720

cag cag ctg ctg aac cag ggc ata cct gtg gcc ccc cac aca act gag      2208
Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu
                725                 730                 735

ccc atg ctg atg gag tac cct gag gct ata act cgc cta gtg aca ggg      2256
Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly
            740                 745                 750

gcc cag agg ccc ccc gac cca gct cct gct cca ctg ggg gcc ccg ggg      2304
Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly
        755                 760                 765
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ctc ccc aat ggc ctc ctt tca gga gat gaa gac ttc tcc tcc att gcg 2352
Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala
770 775 780

gac atg gac ttc tca gcc ctg ctg agt cag atc agc tcc taa 2394
Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser *
785 790 795

<210> 14
<211> 797
<212> PRT
<213> Aequorea victoria and human

<400> 14
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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

Gly Leu Arg Ser Arg Ala Met Asp Glu Leu Phe Pro Leu Ile Phe Pro
245 250 255

Ala Glu Pro Ala Gln Ala Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln
260 265 270

Pro Lys Gln Arg Gly Met Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser
275 280 285

Ala Gly Ser Ile Pro Gly Glu Arg Ser Thr Asp Thr Thr Lys Thr His
290 295 300

Pro Thr Ile Lys Ile Asn Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile
305 310 315 320

Ser Leu Val Thr Lys Asp Pro Pro His Arg Pro His Pro His Glu Leu
325 330 335

Val Gly Lys Asp Cys Arg Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro
340 345 350

Asp Arg Cys Ile His Ser Phe Gln Asn Leu Gly Ile Gln Cys Val Lys
355 360 365

Lys Arg Asp Leu Glu Gln Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn
370 375 380

Asn Pro Phe Gln Val Pro Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu
385 390 395 400

Asn Ala Val Arg Leu Cys Phe Gln Val Thr Val Arg Asp Pro Ser Gly
405 410 415

Arg Pro Leu Arg Leu Pro Pro Val Leu Pro His Pro Ile Phe Asp Asn
420 425 430

Arg Ala Pro Asn Thr Ala Glu Leu Lys Ile Cys Arg Val Asn Arg Asn
435 440 445

Ser Gly Ser Cys Leu Gly Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys
450 455 460

Val Gln Lys Glu Asp Ile Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu
465 470 475 480

Ala Arg Gly Ser Phe Ser Gln Ala Asp Val His Arg Gln Val Ala Ile
485 490 495

Val Phe Arg Thr Pro Pro Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val
500 505 510

Arg Val Ser Met Gln Leu Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu
515 520 525

Pro Met Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg Ile
530 535 540

Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys
545 550 555 560

Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg
565 570 575

Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala Pro
580 585 590

Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu
595 600 605

Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala
610 615 620

Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala
625 630 635 640

Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val
645 650 655

Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro
660 665 670

Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln
675 680 685

Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr
690 695 700

Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe
705 710 715 720

Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu
725 730 735

Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly
740 745 750

Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly
755 760 765

Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala
770 775 780

Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
785 790 795

<210> 15

<211> 2394

<212> DNA

<213> Aequorea victoria and human

<220>
<221> CDS
<222> (1)...(2394)

<400> 15

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1 5 10 15

tct ggc ccc tat gtg gag atc att gag cag ccc aag cag cgg ggc atg 96
Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
20 25 30

cgc ttc cgc tac aag tgc gag ggg cgc tcc gcg ggc agc atc cca ggc 144
Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
35 40 45

gag agg agc aca gat acc acc aag acc cac ccc acc atc aag atc aat 192
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
50 55 60

ggc tac aca gga cca ggg aca gtg cgc atc tcc ctg gtc acc aag gac 240
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65 70 75 80

cct cct cac cgg cct cac ccc cac gag ctt gta gga aag gac tgc cgg 288
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
85 90 95

gat ggc ttc tat gag gct gag ctc tgc ccg gac cgc tgc atc cac agt 336
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
100 105 110

ttc cag aac ctg gga atc cag tgt gtg aag aag cgg gac ctg gag cag 384
Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
115 120 125

gct atc agt cag cgc atc cag acc aac aac aac ccc ttc caa gtt cct 432

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Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
    130                 135                 140

ata gaa gag cag cgt ggg gac tac gac ctg aat gct gtg cgg ctc tgc      480
Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145                 150                 155                 160

ttc cag gtg aca gtg cgg gac cca tca ggc agg ccc ctc cgc ctg ccg      528
Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
                165                 170                 175

cct gtc ctt cct cat ccc atc ttt gac aat cgt gcc ccc aac act gcc      576
Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
            180                 185                 190

gag ctc aag atc tgc cga gtg aac cga aac tct ggc agc tgc ctc ggt      624
Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
        195                 200                 205

ggg gat gag atc ttc cta ctg tgt gac aag gtg cag aaa gag gac att      672
Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
    210                 215                 220

gag gtg tat ttc acg gga cca ggc tgg gag gcc cga ggc tcc ttt tcg      720
Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225                 230                 235                 240

caa gct gat gtg cac cga caa gtg gcc att gtg ttc cgg acc cct ccc      768
Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
                245                 250                 255

tac gca gac ccc agc ctg cag gct cct gtg cgt gtc tcc atg cag ctg      816
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
            260                 265                 270

cgg cgg cct tcc gac cgg gag ctc agt gag ccc atg gaa ttc cag tac      864
Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
        275                 280                 285

ctg cca gat aca gac gat cgt cac cgg att gag gag aaa cgt aaa agg      912
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Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
290 295 300

aca tat gag acc ttc aag agc atc atg aag aag agt cct ttc agc gga 960
Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305 310 315 320

ccc acc gac ccc cgg cct cca cct cga cgc att gct gtg cct tcc cgc 1008
Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
325 330 335

agc tca gct tct gtc ccc aag cca gca ccc cag ccc tat ccc ttt acg 1056
Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
340 345 350

tca tcc ctg agc acc atc aac tat gat gag ttt ccc acc atg gtg ttt 1104
Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
355 360 365

cct tct ggg cag atc agc cag gcc tcg gcc ttg gcc ccg gcc cct ccc 1152
Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
370 375 380

caa gtc ctg ccc cag gct cca gcc cct gcc cct gct cca gcc atg gta 1200
Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385 390 395 400

tca gct ctg gcc cag gcc cca gcc cct gtc cca gtc cta gcc cca ggc 1248
Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
405 410 415

cct cct cag gct gtg gcc cca cct gcc ccc aag ccc acc cag gct ggg 1296
Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
420 425 430

gaa gga acg ctg tca gag gcc ctg ctg cag ctg cag ttt gat gat gaa 1344
Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
435 440 445

gac ctg ggg gcc ttg ctt ggc aac agc aca gac cca gct gtg ttc aca 1392

Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
450 455 460

gac ctg gca tcc gtc gac aac tcc gag ttt cag cag ctg ctg aac cag 1440
Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465 470 475 480

ggc ata cct gtg gcc ccc cac aca act gag ccc atg ctg atg gag tac 1488
Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
485 490 495

cct gag gct ata act cgc cta gtg aca ggg gcc cag agg ccc ccc gac 1536
Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
500 505 510

cca gct cct gct cca ctg ggg gcc ccg ggg ctc ccc aat ggc ctc ctt 1584
Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
515 520 525

tca gga gat gaa gac ttc tcc tcc att gcg gac atg gac ttc tca gcc 1632
Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
530 535 540

ctg ctg agt cag atc agc tcc ttg gat cca ccg gtc gcc acc atg gtg 1680
Leu Leu Ser Gln Ile Ser Ser Leu Asp Pro Pro Val Ala Thr Met Val
545 550 555 560

agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc gag 1728
Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
565 570 575

ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag ggc 1776
Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly
580 585 590

gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc acc 1824
Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
595 600 605

acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctg acc 1872

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr
610 615 620

tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag cag cac 1920
Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His
625 630 635 640

gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc acc 1968
Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr
645 650 655

atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg aag 2016
Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys
660 665 670

ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac 2064
Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp
675 680 685

ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac 2112
Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr
690 695 700

aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac ggc atc 2160
Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
705 710 715 720

aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag 2208
Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln
725 730 735

ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg 2256
Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
740 745 750

ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc aaa 2304
Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys
755 760 765

gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc 2352

Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
770 775 780

gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa 2394
Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys *
785 790 795

<210> 16
<211> 797
<212> PRT
<213> Aequorea victoria and human

<400> 16
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Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
20 25 30
Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
35 40 45
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
50 55 60
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65 70 75 80
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
85 90 95
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser
100 105 110
Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
115 120 125
Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe Gln Val Pro
130 135 140
Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145 150 155 160
Phe Gln Val Thr Val Arg Asp Pro Ser Gly Arg Pro Leu Arg Leu Pro
165 170 175
Pro Val Leu Pro His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
180 185 190
Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
195 200 205

Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
210 215 220
Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225 230 235 240
Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
245 250 255
Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
260 265 270
Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
275 280 285
Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
290 295 300
Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly
305 310 315 320
Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg
325 330 335
Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr
340 345 350
Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe
355 360 365
Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro
370 375 380
Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val
385 390 395 400
Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly
405 410 415
Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly
420 425 430
Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu
435 440 445
Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr
450 455 460
Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln
465 470 475 480
Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr
485 490 495
Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp
500 505 510
Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu
515 520 525

Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
530 535 540

Leu Leu Ser Gln Ile Ser Ser Leu Asp Pro Pro Val Ala Thr Met Val
545 550 555 560

Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
565 570 575

Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly
580 585 590

Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
595 600 605

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr
610 615 620

Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His
625 630 635 640

Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr
645 650 655

Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys
660 665 670

Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp
675 680 685

Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr
690 695 700

Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
705 710 715 720

Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln
725 730 735

Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
740 745 750

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys
755 760 765

Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
770 775 780

Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
785 790 795

<210> 17

<211> 2757

<212> DNA

<213> Aequorea victoria and human

<220>
<221> CDS
<222> (1)...(2757)

<400> 17

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gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc 384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac 432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac 480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc 528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc 576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg 624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc 672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag tcc 720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225 230 235 240

gga ctc aga tct cga ggc aag atg gct gac ccg gct gcg ggg ccg ccg 768
Gly Leu Arg Ser Arg Gly Lys Met Ala Asp Pro Ala Ala Gly Pro Pro
245 250 255

ccg agc gag ggc gag gag agc acc gtg cgc ttc gcc cgc aaa ggc gcc 816
Pro Ser Glu Gly Glu Glu Ser Thr Val Arg Phe Ala Arg Lys Gly Ala
260 265 270

ctc cgg cag aag aac gtg cat gag gtc aag aac cac aaa ttc acc gcc 864
Leu Arg Gln Lys Asn Val His Glu Val Lys Asn His Lys Phe Thr Ala
275 280 285

cgc ttc ttc aag cag ccc acc ttc tgc agc cac tgc acc gac ttc atc 912
Arg Phe Phe Lys Gln Pro Thr Phe Cys Ser His Cys Thr Asp Phe Ile
290 295 300

tgg ggc ttc ggg aag cag gga ttc cag tgc caa gtt tgc tgc ttt gtg 960
Trp Gly Phe Gly Lys Gln Gly Phe Gln Cys Gln Val Cys Cys Phe Val
305 310 315 320

gtg cac aag cgg tgc cat gaa ttt gtc aca ttc tcc tgc cct ggc gct 1008
Val His Lys Arg Cys His Glu Phe Val Thr Phe Ser Cys Pro Gly Ala
325 330 335

gac aag ggt cca gcc tcc gat gac ccc cgc agc aaa cac aag ttt aag 1056
Asp Lys Gly Pro Ala Ser Asp Asp Pro Arg Ser Lys His Lys Phe Lys
340 345 350

atc cac acg tac tcc agc ccc acg ttt tgt gac cac tgt ggg tca ctg 1104
Ile His Thr Tyr Ser Ser Pro Thr Phe Cys Asp His Cys Gly Ser Leu
355 360 365

ctg tat gga ctc atc cac cag ggg atg aaa tgt gac acc tgc atg atg 1152
Leu Tyr Gly Leu Ile His Gln Gly Met Lys Cys Asp Thr Cys Met Met
370 375 380

aat gtg cac aag cgc tgc gtg atg aat gtt ccc agc ctg tgt ggc acg 1200
Asn Val His Lys Arg Cys Val Met Asn Val Pro Ser Leu Cys Gly Thr
385 390 395 400

gac cac acg gag cgc cgc ggc cgc atc tac atc cag gcc cac atc gac 1248
Asp His Thr Glu Arg Arg Gly Arg Ile Tyr Ile Gln Ala His Ile Asp
405 410 415

agg gac gtc ctc att gtc ctc gta aga gat gct aaa aac ctt gta cct 1296
Arg Asp Val Leu Ile Val Leu Val Arg Asp Ala Lys Asn Leu Val Pro
420 425 430

atg gac ccc aat ggc ctg tca gat ccc tac gta aaa ctg aaa ctg att 1344
Met Asp Pro Asn Gly Leu Ser Asp Pro Tyr Val Lys Leu Lys Leu Ile
435 440 445

ccc gat ccc aaa agt gag agc aaa cag aag acc aaa acc atc aaa tgc 1392
Pro Asp Pro Lys Ser Glu Ser Lys Gln Lys Thr Lys Thr Ile Lys Cys
450 455 460

tcc ctc aac cct gag tgg aat gag aca ttt aga ttt cag ctg aaa gaa 1440
Ser Leu Asn Pro Glu Trp Asn Glu Thr Phe Arg Phe Gln Leu Lys Glu
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tcg gac aaa gac aga aga ctg tca gta gag att tgg gat tgg gat ttg 1488
Ser Asp Lys Asp Arg Arg Leu Ser Val Glu Ile Trp Asp Trp Asp Leu
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Thr Ser Arg Asn Asp Phe Met Gly Ser Leu Ser Phe Gly Ile Ser Glu
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ctt cag aag gcc agt gtt gat ggc tgg ttt aag tta ctg agc cag gag 1584
Leu Gln Lys Ala Ser Val Asp Gly Trp Phe Lys Leu Leu Ser Gln Glu
515 520 525

gaa ggc gag tac ttc aat gtg cct gtg cca cca gaa gga agt gag gcc 1632
Glu Gly Glu Tyr Phe Asn Val Pro Val Pro Pro Glu Gly Ser Glu Ala
530 535 540

aat gaa gaa ctg cgg cag aaa ttt gag agg gcc aag atc agt cag gga 1680
Asn Glu Glu Leu Arg Gln Lys Phe Glu Arg Ala Lys Ile Ser Gln Gly
545 550 555 560

acc aag gtc ccg gaa gaa aag acg acc aac act gtc tcc aaa ttt gac 1728
Thr Lys Val Pro Glu Glu Lys Thr Thr Asn Thr Val Ser Lys Phe Asp
565 570 575

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Asn Asn Gly Asn Arg Asp Arg Met Lys Leu Thr Asp Phe Asn Phe Leu
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atg gtg ctg ggg aaa ggc agc ttt ggc aag gtc atg ctt tca gaa cga 1824
Met Val Leu Gly Lys Gly Ser Phe Gly Lys Val Met Leu Ser Glu Arg
595 600 605

aaa ggc aca gat gag ctc tat gct gtg aag atc ctg aag aag gac gtt 1872
Lys Gly Thr Asp Glu Leu Tyr Ala Val Lys Ile Leu Lys Lys Asp Val
610 615 620

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Val Ile Gln Asp Asp Asp Val Glu Cys Thr Met Val Glu Lys Arg Val
625 630 635 640

ttg gcc ctg cct ggg aag ccg ccc ttc ctg acc cag ctc cac tcc tgc 1968
Leu Ala Leu Pro Gly Lys Pro Pro Phe Leu Thr Gln Leu His Ser Cys
645 650 655

ttc cag acc atg gac cgc ctg tac ttt gtg atg gag tac gtg aat ggg 2016
Phe Gln Thr Met Asp Arg Leu Tyr Phe Val Met Glu Tyr Val Asn Gly
660 665 670

ggc gac ctc atg tat cac atc cag caa gtc ggc cgg ttc aag gag ccc 2064
Gly Asp Leu Met Tyr His Ile Gln Gln Val Gly Arg Phe Lys Glu Pro
675 680 685

cat gct gta ttt tac gct gca gaa att gcc atc ggt ctg ttc ttc tta 2112
His Ala Val Phe Tyr Ala Ala Glu Ile Ala Ile Gly Leu Phe Phe Leu
690 695 700

cag agt aag ggc atc att tac cgt gac cta aaa ctt gac aac gtg atg 2160
Gln Ser Lys Gly Ile Ile Tyr Arg Asp Leu Lys Leu Asp Asn Val Met
705 710 715 720

ctc gat tct gag gga cac atc aag att gcc gat ttt ggc atg tgt aag 2208
Leu Asp Ser Glu Gly His Ile Lys Ile Ala Asp Phe Gly Met Cys Lys
725 730 735

gaa aac atc tgg gat ggg gtg aca acc aag aca ttc tgt ggc act cca 2256
Glu Asn Ile Trp Asp Gly Val Thr Thr Lys Thr Phe Cys Gly Thr Pro
740 745 750

gac tac atc gcc ccc gag ata att gct tat cag ccc tat ggg aag tcc 2304
Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr Gln Pro Tyr Gly Lys Ser
755 760 765

gtg gat tgg tgg gca ttt gga gtc ctg ctg tat gaa atg ttg gct ggg 2352
Val Asp Trp Trp Ala Phe Gly Val Leu Leu Tyr Glu Met Leu Ala Gly
770 775 780

cag gca ccc ttt gaa ggg gag gat gaa gat gaa ctc ttc caa tcc atc 2400
Gln Ala Pro Phe Glu Gly Glu Asp Glu Asp Glu Leu Phe Gln Ser Ile
785 790 795 800

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Met Glu His Asn Val Ala Tyr Pro Lys Ser Met Ser Lys Glu Ala Val
805 810 815

gcc atc tgc aaa ggg ctg atg acc aaa cac cca ggc aaa cgt ctg ggt 2496
Ala Ile Cys Lys Gly Leu Met Thr Lys His Pro Gly Lys Arg Leu Gly
820 825 830

tgt gga cct gaa ggc gaa cgt gat atc aaa gag cat gca ttt ttc cgg 2544
Cys Gly Pro Glu Gly Glu Arg Asp Ile Lys Glu His Ala Phe Phe Arg
835 840 845

tat att gat tgg gag aaa ctt gaa cgc aaa gag atc cag ccc cct tat 2592
Tyr Ile Asp Trp Glu Lys Leu Glu Arg Lys Glu Ile Gln Pro Pro Tyr
850 855 860

aag cca aaa gct aga gac aag aga gac acc tcc aac ttc gac aaa gag 2640
Lys Pro Lys Ala Arg Asp Lys Arg Asp Thr Ser Asn Phe Asp Lys Glu
865 870 875 880

ttc acc aga cag cct gtg gaa ctg acc ccc act gat aaa ctc ttc atc 2688
Phe Thr Arg Gln Pro Val Glu Leu Thr Pro Thr Asp Lys Leu Phe Ile
885 890 895

atg aac ttg gac caa aat gaa ttt gct ggc ttc tct tat act aac cca 2736
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900 905 910

gag ttt gtc att aat gtg tag 2757
Glu Phe Val Ile Asn Val *
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            20                  25                  30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
225                 230                 235                 240
Gly Leu Arg Ser Arg Gly Lys Met Ala Asp Pro Ala Ala Gly Pro Pro
                245                 250                 255
Pro Ser Glu Gly Glu Glu Ser Thr Val Arg Phe Ala Arg Lys Gly Ala
            260                 265                 270
Leu Arg Gln Lys Asn Val His Glu Val Lys Asn His Lys Phe Thr Ala
        275                 280                 285
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Arg Phe Phe Lys Gln Pro Thr Phe Cys Ser His Cys Thr Asp Phe Ile
290 295 300

Trp Gly Phe Gly Lys Gln Gly Phe Gln Cys Gln Val Cys Cys Phe Val
305 310 315 320

Val His Lys Arg Cys His Glu Phe Val Thr Phe Ser Cys Pro Gly Ala
325 330 335

Asp Lys Gly Pro Ala Ser Asp Asp Pro Arg Ser Lys His Lys Phe Lys
340 345 350

Ile His Thr Tyr Ser Ser Pro Thr Phe Cys Asp His Cys Gly Ser Leu
355 360 365

Leu Tyr Gly Leu Ile His Gln Gly Met Lys Cys Asp Thr Cys Met Met
370 375 380

Asn Val His Lys Arg Cys Val Met Asn Val Pro Ser Leu Cys Gly Thr
385 390 395 400

Asp His Thr Glu Arg Arg Gly Arg Ile Tyr Ile Gln Ala His Ile Asp
405 410 415

Arg Asp Val Leu Ile Val Leu Val Arg Asp Ala Lys Asn Leu Val Pro
420 425 430

Met Asp Pro Asn Gly Leu Ser Asp Pro Tyr Val Lys Leu Lys Leu Ile
435 440 445

Pro Asp Pro Lys Ser Glu Ser Lys Gln Lys Thr Lys Thr Ile Lys Cys
450 455 460

Ser Leu Asn Pro Glu Trp Asn Glu Thr Phe Arg Phe Gln Leu Lys Glu
465 470 475 480

Ser Asp Lys Asp Arg Arg Leu Ser Val Glu Ile Trp Asp Trp Asp Leu
485 490 495

Thr Ser Arg Asn Asp Phe Met Gly Ser Leu Ser Phe Gly Ile Ser Glu
500 505 510

Leu Gln Lys Ala Ser Val Asp Gly Trp Phe Lys Leu Leu Ser Gln Glu
515 520 525

Glu Gly Glu Tyr Phe Asn Val Pro Val Pro Pro Glu Gly Ser Glu Ala
530 535 540

Asn Glu Glu Leu Arg Gln Lys Phe Glu Arg Ala Lys Ile Ser Gln Gly
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Thr Lys Val Pro Glu Glu Lys Thr Thr Asn Thr Val Ser Lys Phe Asp
565 570 575

Asn Asn Gly Asn Arg Asp Arg Met Lys Leu Thr Asp Phe Asn Phe Leu
580 585 590

Met Val Leu Gly Lys Gly Ser Phe Gly Lys Val Met Leu Ser Glu Arg
595 600 605

Lys Gly Thr Asp Glu Leu Tyr Ala Val Lys Ile Leu Lys Lys Asp Val
610 615 620

Val Ile Gln Asp Asp Asp Val Glu Cys Thr Met Val Glu Lys Arg Val
625 630 635 640

Leu Ala Leu Pro Gly Lys Pro Pro Phe Leu Thr Gln Leu His Ser Cys
645 650 655

Phe Gln Thr Met Asp Arg Leu Tyr Phe Val Met Glu Tyr Val Asn Gly
660 665 670

Gly Asp Leu Met Tyr His Ile Gln Gln Val Gly Arg Phe Lys Glu Pro
675 680 685

His Ala Val Phe Tyr Ala Ala Glu Ile Ala Ile Gly Leu Phe Phe Leu
690 695 700

Gln Ser Lys Gly Ile Ile Tyr Arg Asp Leu Lys Leu Asp Asn Val Met
705 710 715 720

Leu Asp Ser Glu Gly His Ile Lys Ile Ala Asp Phe Gly Met Cys Lys
725 730 735

Glu Asn Ile Trp Asp Gly Val Thr Thr Lys Thr Phe Cys Gly Thr Pro
740 745 750

Asp Tyr Ile Ala Pro Glu Ile Ile Ala Tyr Gln Pro Tyr Gly Lys Ser
755 760 765

Val Asp Trp Trp Ala Phe Gly Val Leu Leu Tyr Glu Met Leu Ala Gly
770 775 780

Gln Ala Pro Phe Glu Gly Glu Asp Glu Asp Glu Leu Phe Gln Ser Ile
785 790 795 800

Met Glu His Asn Val Ala Tyr Pro Lys Ser Met Ser Lys Glu Ala Val
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Ala Ile Cys Lys Gly Leu Met Thr Lys His Pro Gly Lys Arg Leu Gly
820 825 830

Cys Gly Pro Glu Gly Glu Arg Asp Ile Lys Glu His Ala Phe Phe Arg
835 840 845

Tyr Ile Asp Trp Glu Lys Leu Glu Arg Lys Glu Ile Gln Pro Pro Tyr
850 855 860

Lys Pro Lys Ala Arg Asp Lys Arg Asp Thr Ser Asn Phe Asp Lys Glu
865 870 875 880

Phe Thr Arg Gln Pro Val Glu Leu Thr Pro Thr Asp Lys Leu Phe Ile
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Met Asn Leu Asp Gln Asn Glu Phe Ala Gly Phe Ser Tyr Thr Asn Pro
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Glu Phe Val Ile Asn Val
915